STUDIES ON SERUM CHOLESTEROL AND OTHER CORONARY HEART DISEASE PRECURSORS IN CHILDHOOD

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STATEMENT OF ORIGINALITY

All these studies were planned and performed by myself except where indicated in the Acknowledgements. All the individual chapters from 4 to 12 have been submitted for publication or have been published. A list of publications to date has been included after the bibliography.

INTRODUCTION

The change in the pattern of diseases affecting children in industrialised countries during the last century has caused a shift in emphasis from curative to preventive paediatrics.

Coronary heart disease (CHD) is one of the major causes of morbidity and mortality in adulthood. Its origins have been shown by epidemiological studies to lie in facets of the Western life-style which are biologically disadvantageous. By the time CHD becomes symptomatic, primary prevention is too late; but what is the evidence that by starting these measures in early life benefit would result?

The studies described in this thesis were planned to determine whether early deviations from the biological norm occur during childhood for factors known to be associated with CHD in adult life. These factors include blood pressure, personality type, and serum cholesterol. The concentration of cholesterol in serum was chosen because its abnormalities are unequivocally associated with an excess risk of CHD, and because it provides a model for analysis of both genetic and environmental nutritional influences.

Although there has been an enormous amount of research into the various influences on serum cholesterol, there is very little data about the subject during the first two years of life when future metabolic homeostatic and nutritional patterns may become established. For that reason a longitudinal study was planned, and the data derived from it was supplemented by cross-sectional studies on older children.

In the first chapter I have presented an over-view of the subject of coronary heart disease (CHD) risk factors and the causal aetiological influences as they may affect children. The subject of CHD and its related research disciplines encompasses very many diverse topics ranging from psychology to immunology, through lipoprotein biochemistry, clinical nutrition, and cardiology. Clearly some of these subjects fall out-with the field of research and discussion of this thesis; and as the studies comprising the thesis themselves range widely in emphasis, fuller discussion has been included in some instances with the subsequent chapter concerned. I have therefore selected for discussion topics relating primarily to serum cholesterol as a measure of CHD risk, including factors such as genetically determined hypercholesterolaemia (HC), nutrition, and vascular evidence for the origins of atherosclerosis in childhood. Blood pressure, activity, and lifestyle, obesity, and smoking, although recognised to be as equally important as HC in mediating CHD risk, have received less emphasis in this context.

In the subsequent chapters are described the studies concerning cord blood screening and the analyses on the influences on serum cholesterol levels in childhood followed by descriptions of the studies which investigated the extent to which children may be identified as carrying biochemical and/or anthropometric markers for excess coronary risk.

ABSTRACT

Studies on normal children were performed to investigate the extent of the influences on serum cholesterol of genetic, perinatal, familial, nutritional, and anthropometric factors through childhood. In addition biochemical evidence by which to identify those children with early markers for coronary heart disease (CHD) was sought.

The samples comprised children selected at birth and followed from three months (n=391) to two years (n=198); 488 four-year-olds; and 235 school children from 8.2 to 17.9 years.

Low density lipoprotein cholesterol (LDL-C) was shown not to be a reliable marker for familial hypercholesterolaemia (FH), with false positive and negative results invalidating the procedure. Adverse maternal and perinatal factors also cause changes to cord serum cholesterol and triglyceride levels, which may produce additional false positive results.

During the second year of life familial factors (the parents' cholesterol level) became important associated influences on the children's cholesterol level, with nutritional and anthropometric factors being not associated then or during school age. However, individual changes in dietary fat and cholesterol were shown to cause large changes in serum cholesterol during infancy, with evidence for a maturation in the child's cholesterol homeostatic mechanism through infancy. Tracking of cholesterol emerged during the second year providing further evidence of biochemical programming under genetic influences.

Screening for hypercholesterolaemia improved in accuracy from age four. Gradations in the extent of the family history of CHD were found to be associated with differences in the two-year-olds' serum total cholesterol, LDL-C, and HDL-C; and concordance of CHD risk factors increased from four years of age to school age.

These results are interpreted as demonstrating that familial influences mediating CHD become detectable in the first few years of life, and additional environmental influences may result in an adult coronary risk profile being identifiable by school age.

CHAPTER 1

CHILDHOOD ANTECEDENTS OF CORONARY HEART DISEASE

Coronary heart disease (CHD) is recognised as one of the major causes of death in developed countries (Keys 1970, Stammler 1970, Editorial 1976a). Australia rates amongst the highest with 36% and 20% of all deaths in men and women respectively in 1976 being a result of CHD (Nat. Heart Foundation 1976). These high values are in spite of a slight but definite decrease in the incidence of CHD mortality which has occurred since the mid-sixties (Figure 1). This decline has been noted both in Australia (Reader 1972, Christie 1974 Craig et al 1978) and the United States (Gordon and Thom 1975, Borhani 1975) and has been variously attributed to improved clinical management of the immediate complications following myocardial infarction, a decline in hypertension, and epidemic fluctuations in the incidence of respiratory diseases. Until a persistent downward trend is achieved, however, much more work must be carried out on the mechanism and prevention of CHD.

The low survival rate after myocardial infarction suggests the need for primary rather than secondary prevention (Roy Coll Phyns London, 1976). As CHD is a progressive disease with a long latent period its origins may be established in early life. If this is so, then primary prevention should be considered to be in the province of the paediatrician (Kannel & Dawber 1972, de Haas 1973).

The Evidence for Precursors of Arteriosclerosis in Childhood

It is well recognised that the incidence of CHD increases with age (Kannel 1976a) and Table 1:1 shows this trend for Australian adults. Autopsy studies by Vihert (1976) found evidence for myocardial

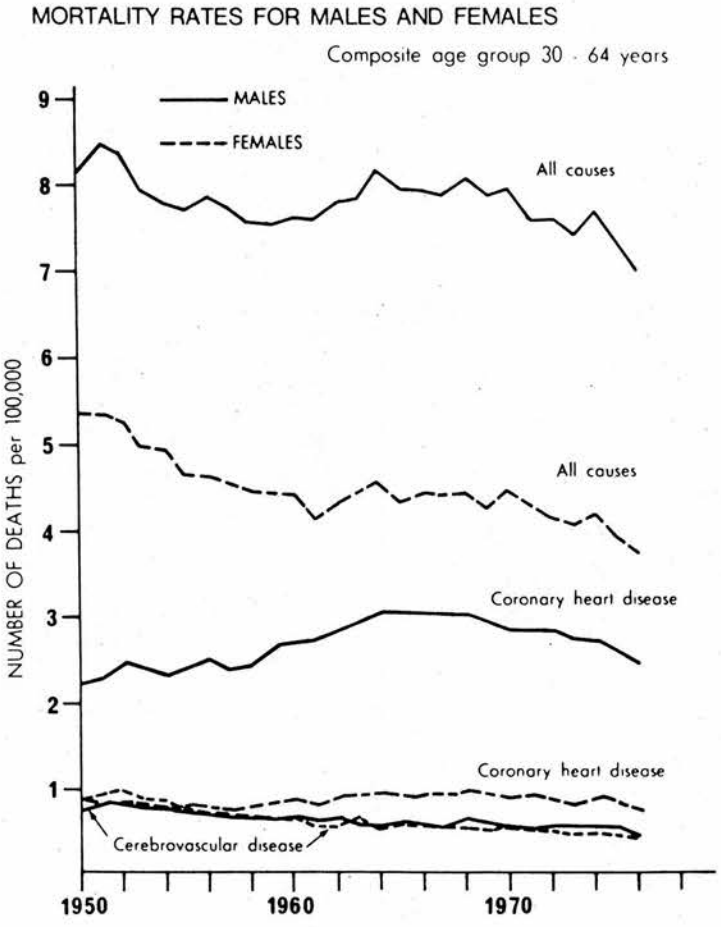

Fig 1. Mortality rates for the Australian population 1950 to 1976 for men and women separately from all causes, coronary heart disease and cerebovascular disease.

TABLE 1:1 Mortality from circulatory disease in Australia 1976.

(*Composite rates are age adjusted to 1961 population age structure).

Deaths rate/100,000	30-64	Age (years) 30-34	35-39	40-44	45-49	50-54	55-59	60-64
Males								
All causes	697*	138.7	196.9	334.5	558.2	916.9	1496.7	2451.3
CHD	247*	9.6	31.8	85.0	198.8	343.0	549.9	969.4
CVD	45*	4.6	7.7	16.2	30.7	62.1	106.8	177.0
All disease	332*	19	48.1	120.1	254.7	454.7	789.4	1309.0
Females								
All causes	574*	75.4	118.6	193.8	312.7	493.2	745.1	1160.7
CHD	73*	3.2	9.1	23.3	39.0	86.4	169.0	317.4
CVD	40*	4.5	12.3	16.1	32.4	47.4	84.5	145.9
All disease	137*	12.1	29.5	50.3	93.8	157.9	292.9	544.5

From National Heart Foundation of Australia. Information Paper No. 10.

CHD Coronary heart disease

CVD Cardiovascular disease

infarction occurring as early as the second decade in 2% males but not until the fourth decade in females. In both sexes this prevalence increased with age.

Because CHD is usually preceded by coronary vascular atherosclerosis with stenosis and calcification (Vihert 1976), its extent in early life may therefore be used as an indicator of likely CHD risk in later life. Studies of arterial wall lesions have shown that fatty streaks are present in the aortas of children as young as three years of age (Holman et al 1958). Coronary artery fatty streaks are rare before the age of ten years, then increase in frequency, being nearly always present after twenty years (Strong and McGill, 1969). In their study of six geographic and ethnic groups in which a wide spectrum of extent of advanced atherosclerosis was found in older people no geographic differences were found in the overall extent of fatty streaking. However a racial difference was found with Blacks having more extensive fatty streaking, even though they developed less extensive advanced lesions. When Black and White populations were considered separately with respect to coronary artery lesions, then populations with extensive fatty streaks in childhood were shown to have more extensive raised atherosclerotic lesions in middle age.

Vanecek (1976) found little difference in the prevalence of fatty streaking in coronary arteries between the populations of five European towns with differing demographic characteristics and CHD incidence. Fatty streaks increased in prevalence from early adolescence but they remained the only arterial wall lesion

in 0.3% of men, compared with 90% who had fibrous plaques after 40 years. Katz et al (1976) found biochemical and physical evidence of lesions intermediate between fatty streaks and advanced plaques but it is speculative as to whether all or only some streaks continue to develop into fibrous plaques (Small 1977).

The evidence therefore suggests that although atherosclerosis may develop from fatty streaks, the degree to which transformation into plaques takes place varies between arterial sites, and amongst racial groups. The extent of fatty streaking in children therefore may not provide an absolute criterion for the risk of developing advanced atherosclerosis. By the age of twenty raised lesions significant to the development of clinical disease have already begun to appear in coronary arteries. Strong and McGill (1969) found 7.22% of 15-19 year olds (Black and White) had raised atherosclerotic lesions. Autopsies of United States soldiers killed in Korea (average age 22 years) revealed 77.3% had atheroma with 5.3% having greater than 90% narrowing of the arteries (Enos et al, 1953). A similar study on United States Vietnam casualties (average age about 22) showed that 45% had some evidence of atherosclerosis with 5% having severe coronary atherosclerosis (McNamara et al, 1971).

These studies show that small infarcts may occur in the first decades, and atheromatous lesions which may lead to CHD in later life, are already present. Information about the factors which contribute to the progression of atherosclerosis through early adult life and which add to the risk for CHD is therefore

necessary for primary preventive measure to be planned.

Serum Cholesterol and CHD Risk

The prevalence of CHD is associated with a population's serum cholesterol levels (Keys, 1970), and this relationship is seen in the relatively homogeneous populations of London (Lewis et al, 1974a,b) and Framingham in the U.S.A. (Kannel et al, 1971; Kannel, 1976a). This association may be demonstrated in presymptomatic subjects from the correlation of exercise ECG changes with hypercholesterolaemia (HC), and in autopsy studies with the extent of coronary vascular disease (Schwartz et al, 1965). HC is common amongst adult populations in Europe (Lewis et al, 1974a; Bjorkstein et al, 1975; Slack et al 1977) and the U.S.A. (Schilling et al, 1964) compared with developing countries (Onitiri et al, 1977). This prevents a clear-cut definition of HC from being made for any particular population (Schwartz and Hill, 1972), for a serum cholesterol level within the observed normal range may be above the biologically optimal limits. Although the lipoprotein electrophoretic pattern itself may not provide greater discriminant value for CHD prediction than the cholesterol concentration alone (Masarei et al, 1971), each of the abnormal lipoprotein phenotypes (excluding Type I) is associated with an increased CHD risk, including Type IV (Salel et al, 1974). Type II familial hypercholesterolaemia (FH) is associated with a particularly high risk of early symptomatic CHD. This level of excess risk is greater than can be explained by the degree of HC alone (Slack 1969), and is also expressed in

other family members (Slack and Nevin 1968). For men with FH the age of death does not correlate with the level of serum cholesterol, although there is strong concordance between affected brothers, which also suggests additional factors operate in transmitting CHD risk in this condition (Heiberg and Slack, 1977).

Apart from the example of familial CHD risk which is seen in FH, there is clear evidence that much of familially transmitted risk is also mediated through HC (Deutscher et al, 1970; Nikkilä and Aro, 1973; Goldstein et al, 1973; Goldstein and Brown, 1975; Förde and Thelle, 1977). However, there is dispute as to whether single gene effects are seen in more than a minority of such families (Patterson and Slack, 1974). Epstein (1976) suggests that this familial aggregation may be due to additional as yet unknown factors, because CHD risk factors overall are not strongly inherited. For example, in men with a history of early parental CHD Scholtz et al (1975) described a significantly increased frequency of Type A behaviour patterns, in addition to higher serum cholesterol and low density : high density lipoprotein cholesterol ratios. These studies have been extended to the children of parents with early CHD. Tamir et al (1972) found that nearly half of the children of young (under 40 years) HC survivors of infarct also had HC, but that all the children of normocholesterolaemic (NC) survivors were NC. Gross and Caplan (1978) found a 16% incidence of HC in preteen children of parents who had had an infarct, and Hennekens et al (1976a) reported a higher mean serum cholesterol level in such children. Dzizinski and Puzyrev (1976) recorded similar findings, but also found that

this effect diminished with increasing age, suggesting that environmental influences confound familial influences in later life. Further evidence for the significance of HC in FH has been described in the abnormal exercise ECG patterns of affected children (James et al, 1975).

Genetic Influences on Serum Cholesterol

Although both monogenic (Namboodiri et al, 1975) and polygenic (Sing and Orr, 1976) effects are recognised to be important influences on serum cholesterol (Carter, 1974), their relative importance in the extent to which they determine the serum cholesterol level in conjunction with other factors such as obesity, activity level, and diet, is quite unclear for the majority of people (Editorial 1977a). Environmental effects mediated through socio-economic status also account for a minor degree of variance between groups (Orr et al, 1975).

Twins provide a genetic model in which to compare heritable versus environmental factors (Pikkarainen et al, 1966). Greater variance occurred for dizygotic (DZ) than monozygotic (MZ) twins' cholesterol; for MZ twins living apart than together; and for DZ twins of different sex than of the same sex (Meyer, 1962). Similar findings have also been reported for free cholesterol, glycerol, and uric acid (Jensen et al, 1965), as well as for various cell surface antigens and enzyme levels, in addition to lipoprotein levels (Heiberg, 1974). Different degrees of variance between the various lipid components exist, with the highest degree of heritability occurring for triglyceride (Christian et al, 1976).

With specific reference to CHD, Feinleib et al (1977) considered that there was evidence for a greater degree of heritability for blood pressure, glucose tolerance, serum uric acid, and triglyceride, than for high or low density lipoprotein cholesterol, to which they suggested the environment contributed to the familial similarity. Rifkind et al (1968) also concluded that environmental rather than genetic factors had greater influence on serum lipid levels. In the Swedish twin study surviving twins of those dying of CHD had a significantly higher incidence of angina, previous infarct, and ECG changes of ischaemia, than control twins, suggesting a genetic basis for the difference (de Faire, 1976). These observations were supported by results from a long-term follow up of those twins originally discordant for signs of CHD, for ten of the 19 MZ male twins developed CHD (Liljefors, 1976).

Family patterns of cholesterol distribution may be confounded by dietary factors. These were excluded in a Kibbutz study in which no correlation was found to exist between children's and parents' cholesterol levels, (Brunner et al, 1971) and in a study on an isolated inbred religious community in the U.S.A. (called H-leut) in which the families did not eat together (Martin et al, 1973). Amongst the H-Leut, patterns of familial correlations for serum cholesterol occurred which were consistent with both a polygenic mode of inheritance as well as with monogenic inheritance confounded by a large environmental factor.

Studies from parents and children living together normally have shown that significant correlations exist (Godfrey et al 1972),

with HC children also being more likely to have HC parents (Morrison et al, 1977a). Mimura (1975) concluded from family studies that there was strong evidence for a significant degree of heritability for cholesterol with sib:sib correlations being similar to parent : children correlations. Similar levels of correlation were described by Johnson et al (1965), being marginally higher between fathers and both sons and daughters than between mothers and daughters. Schaefer et al (1958) reported higher degrees of correlation between mothers and children than for fathers and children of either sex, with the sib:sib correlation being greater than for mothers and children.

Cholesterol Levels and Hypercholesterolaemia in Children

In populations with a high prevalence of HC in adults it is important to define the extent of HC in children (Fredrickson and Breslow, 1973; Neil et al, 1977). Much data has accumulated on cholesterol levels in children from the several large American Community studies (Berenson et al, 1974; Frerichs et al, 1976; Levy et al, 1976; Srinivasan et al, 1976; Morrison et al 1977b, 1978; de Groot et al, 1977) as well from N. Europe (Dyerberg and Hjörne, 1973; Strunge and Trostman, 1976, 1978; Askevold et al, 1978), and Australia (Godfrey et al, 1972; Court and Dunlop, 1975; Hickie et al, 1975b, 1977). These data have been summarised in table 1:2 and the triglyceride results in table 1:3. Although cut-off points may be defined from these studies, the role of screening for HC in children is not clear. This is partly because of the conflicting evidence concerning its validity at different ages

Table 1:2 Serum cholesterol levels in children as reported by various authors with the chosen cut-off points.

Author -Study	Age (years)	Sex	Mean S.D. mmol/L	Cutt off mmol/L	% Above cut off
Hickie (1975)	6-10	m & f	4.24 ± 0.90	-	-
Hickie et al (1977)	14	m & f	4.87	5.2	26%
				6.2	4%
Court & Dunlop (1975)	11-19	m	4.65	-	-
Godfrey et al (1972)	6	m & f	4.14	6.2	18%
Whyte & Yee (1958)	6-10	m & f	4.24 ± 0.90	-	-
	11-15	m & f	4.80 ± 1.30	-	-
	6-10	m & f	3.70 ± 0.75	-	-
	11-15	m & f	3.47 ± 0.98	-	-
Askevold et al (1978)	13-14	f	4.63 ± 0.09	-	-
		m	4.52 ± 0.08	-	-
	15-16	f	4.76 ± 0.06	-	-
		m	4.40 ± 0.10	-	-
Strunge & Trostmann (1978)	8-18	f	4.92 ± 0.75	-	-
		m	4.73 ± 0.80	-	-

Table 1:2 Contd:

Author -Study	Age (years)	Sex	Mean S.D. mmol/L	Cut off mmol/L	% above cut off
Dyerberg & Hjorne (1973)	6-15	m & f	5.28 ± 0.82	-	-
Morrison et al (1978)	6-11	f	4.18 ± 0.56	5.25	5%
		m	4.16 ± 0.62	-	-
Lauer & Muscatine (1975)	6-18	m & f	4.71 ± 0.75	5.2	24%
Berenson et al (1974)	3-16	m & f	4.40 ± 1.03	-	-
This study	6months	f	4.39 ± 0.95	6.13	3%
		m	4.28 ± 0.84	5.72	
	2	f	4.20 ± 0.68	5.69	"
		m	4.08 ± 0.78	5.33	"
	4	f	4.69 ± 0.83	6.29	"
		m	4.70 ± 0.82	6.24	"
	8-18	f	4.95 ± 0.97	7.02	"
		m	4.92 ± 0.96	7.28	"

Table 1:3 Serum triglyceride levels in children as recorded by various authors.

Author -Study	Country	Age (years)	Sex	Mean S.D. mmol/L
Hickie et al (1977)	Australia	10-16	m	0.88 ± 0.35
				0.71 ± 0.48
Court & Dunlop (1975)	Melbourne	11-19	m	0.95
Askevold et al (1978)	Norway	13-14	f	1.26 ± 0.05
		15-16		1.20 ± 0.05
		13-14	m	1.15 ± 0.06
		15-16		1.32 ± 0.06
Strunge & Trostman (1978)	Denmark	8-17	m & f	0.83 ± 0.29
				0.75 ± 0.28
Dyerberg & Hjorne (1973)	Denmark	6-15	f	0.81 ± 0.26
		16-20		1.07 ± 0.39
		6-15	m	0.74 ± 0.31
		16-20		0.91 ± 0.26
This study	Adelaide	4	f	0.92 ± 0.29
			m	0.94 ± 0.36
		8-18	f	1.04 ± 0.48
			m	0.98 ± 0.42

from birth to teenage, and also because no long-term beneficial effects in terms of reduced CHD morbidity have yet been shown to occur from dietary and therapeutic intervention which is acceptable to a child (Lloyd et al 1975). Leonard et al (1976), found random screening of a hospital population to be unrewarding and diagnostically confusing, with few cases of FH being detected. This was in part due to the grey zone of cholesterol levels at the upper end of the normal range, but the lower end of the range for FH. (Leonard et al 1977). However, screening children of families with a history of CHD may be more productive (Chase et al 1974).

Diet and Coronary Heart Disease

Perhaps because dietary habits and nutritional practices are an integral part of everyone's life style, objective scientific discussion on this important and fascinating area is tinged with more emotive acrimony than would seem justified (Mann 1977; Glueck et al 1978). This is clear evidence from extensive epidemio-logical studies that nutritional factors are of major importance in explaining the difference between CHD prevalence between countries (Bronte-Stewart et al 1955; Keys 1970; Truswell 1976) with diet-related differences in serum cholesterol levels between populations mediating CHD risk. Smaller studies have shown that it is changes in dietary habits rather than other measurements of Westernisation that induce the changes in serum cholesterol (Ostwald and Gebre-Medhin 1978). These results have led to authoritative statements concerning suitable dietary changes for individuals and communities (McBean and Speckman 1974, Shaper and Marr 1977). However the extent to which these recommendations are justified has led to divergence of opinion, with the Sub Committee on Atherosclerosis

of the American Heart Association (Mitchell et al 1972), and the Committee on Nutrition of the American Paediatric Association (Filer 1972), stating that such dietary changes are unjustified.

What is the basis for the continuing debate on this issue (Clarke 1974; Reiser 1978; Glueck and Connor 1978)? Accepting that Third World countries have a lower CHD prevalence than First World countries, and that a monotonous low fat, high unrefined carbohydrate diet is the norm throughout these countries, can one extrapolate these findings to deduce a meaningful interpretation relevant to our society? Studies from rural Africa, Asia, and New Guinea (Whyte and Yee 1958; Ho and Chan 1975; Onitiri et al 1977; Glueck and Connor 1978), have shown clear differences in the serum cholesterol levels in these populations compared to inhabitants of Northern European cities (Lewis et al 1974a; Olsson 1974; Slack et al 1977).

Several inter-related factors probably account for the differences in serum cholesterol levels between such populations. The long term effects on body composition of a low energy, low fat, high carbohydrate diet; and the almost invariable presence of 'confounding variables' comprising differences in lifestyle, day to day stress, smoking, and physical activity - each of which may have subtle but profound effects on CHD risk.

Strong positive correlations are present between CHD incidence and protein and fat intake, being negative for starch and vegetable protein (Connor and Connor 1972). Differences in the consumption of dairy products has also been shown to correlate with significant differences in CHD rates between Northern and Southern regions in Belgium (Joosens et al 1977), and Londoners with a high cereal

fibre intake (and independently those with a higher food energy intake) had a lower CHD morbidity than others (Morris et al 1977).

However when samples in a nutritionally homogeneous population are compared no relationship between nutritional variables and the serum cholesterol may appear (Hitchcock and Gracey 1977; Frank et al 1978; Weidman et al 1978), giving rise to controversy as to the magnitude of the effect of saturated fats on serum cholesterol in healthy people (Reiser 1973). Comparison between vegetarians and people on a mixed diet from the same community show clear diet-related effects on cholesterol, with the vegetarians having lower levels (Sacks et al 1975; Simons et al 1978). Substitution of fish for cheese in lacto-ovo-vegetarian monks and nuns resulted in a fall in both serum cholesterol and triglyceride (von Lossonczy et al 1978).

Nutritional studies are perhaps easier to control during infancy, when milk volumes and composition are easily measured. Alterations in the quantity and type of liquid in milk ingested are related to changes in samples' serum cholesterol levels (Fomon and Bartels 1960; Sweeney et al 1961), and to breast fed babies mothers' dietary fat (Potter and Nestel 1976a). Although there is no effect on later serum cholesterol related to early exposure to a polyunsaturated fatty acid (pufa) diet (Friedman and Goldberg 1975), children with FH may be maintained NC on a pufa diet (Glueck and Tsang 1972). These diet: cholesterol inter-relations become clear from metabolic studies which showed that 91% of the total variance in levels of serum cholesterol could be explained by the dietary intake of saturated fatty acids (particularly myristic and palmitic acid), pufa, and dietary

cholesterol (Hegsted et al 1965). Sixty seven% of the variance may be accounted for by changes in myristic acid intake alone; dietary cholesterol relates linearly to serum cholesterol when the daily intake is below 300-600 mg, with 100 mg causing approximately a 5% rise in serum cholesterol; total daily dietary fat providing between 22 to 40% of food energy had no influence on serum cholesterol. Individual week-to-week variations of around 5%, and superimposed seasonal fluctuations on serum cholesterol (Warnick and Albers 1976), provide further confounding variables in community studies of homogeneous samples in which the individuals are ingesting more than the threshold amount of dietary cholesterol (600mg). Thus not only do the variations in diet between indivuduals exert a lesser effect than random fluctuations, but a sample size of over 100 would be needed for there to be an 80% change of detecting a difference at the 5% level of significance (Berwick 1978).

In summary, nutritional factors are of importance both for a population and to the individual, particularly those with a poorly regulated feedback system of turn-off of hepatic cholesterol production and/or increase in endogenous sterol excretion in the face of a high dietary cholesterol load. Furthermore, the overall effect on serum cholesterol of a highly saturated fatty acid, high fat diet may be causally related to the differences in CHD prevalence between populations of radically different nutritional habits. In addition the effects of regular hard physical exertion, the absence of smoking, and being lean, fit, and normotensive, may all add to these differences in morbidity from cardiovascular disease.

Clustering Risk Factors for CHD

The role of HC as a major causal aetiological agent in the

development of CHD may be exaggerated by the presence of other risk factors, in particular smoking, hypertension, and type A personality, each of which independently relate to CHD (Brand et al 1976). Studies of young (less than 40 years) male survivors of an infarct from nine countries (Dolder and Oliver 1975), showed a high prevalence of smoking and HC, with in addition hypertension, obesity, and hypertriglyceridaemia. In comparative studies, the difference in the CHD rate between Edinburgh and Stockholm can be explained by, and thus draws attention to the importance of, the high levels of smoking, BP, and triglyceride in Scotland (Editorial 1976b), with these factors correlating with the extent of coronary vessel lesions (Kagan 1976).

In longitudinal studies the risk of developing clinical evidence of CHD has been shown to relate strongly to the presence of the factors which make up the coronary profile (Kannel et al 1967a; Kannel et al 1975). The importance of these risk factors may be seen from studies of young people and women affected by CHD (Mulcahy et al 1967; Oliver 1974; Mann et al 1975), the inference being that these factors would have to have been particularly deleterious to produce effects at that age and in females. Clustering of these risk factors may occur through causal association - such as hypertriglyceridaemia, abnormal glucose tolerance, and obesity (Albrink and Meigs 1964), but there is further evidence that otherwise unrelated risk factors also occur together more commonly than by chance alone (Morton et al 1977).

Hypertension

Hypertension increases the risk of CHD by accelerating atherosclerosis (Fries 1969; Stammler 1974; Matova and Vihert 1976),

with the average duration of life from the onset of hypertension in adults being 20 years, and 10-14 year old hypertensives having a mean survival rate of 21 years (Loggie 1969; Heyden et al 1969). Blood pressure (BP) increases with age in both sexes (Londe 1976), and hypertension as defined by a systolic and/or diastolic blood pressure greater than 140 and 90 mm Hg respectively occurs in children at a frequency of from 1.4 to 21% (Garfunkel 1971; Voors et al 1976). Essential hypertension, however, should not be regarded as a childhood disease until organic disease has been excluded. For example Masland et al (1956), found that only one of the 25 adolescents they found to be hypertensive could be diagnosed as having essential hypertension, the rest having organic disease. Loggie (1969), and Singh and Page (1967), found that 20% and 35% respectively of hypertensive adolescents had essential hypertension, the remainder having hypertension secondary to organic disease. Table 1:4 summarises the cut-off levels used, and the percentages of children above these levels for these studies. Persistent marginal hypertension in early life may be a precursor to essential hypertension later in life if tracking of blood pressure occurs from childhood (Zinner et al 1975). Kass et al (1977), described positive correlations between successive blood pressure measurements on the same cohort of Boston children aged 2-14 years over an eight year period. They also found that this correlation tended to increase as the children grew older, suggesting that the degree of tracking strengthened. This observation was also reported by Hennekens et al (1977) in Welsh children 5-19 years old. Tracking of blood pressure was also found as early as 5-9 weeks (initial reading 4-6 days) by de Sweit

Table 1:4 Percentage of children above the defined upper normal limit for blood pressure as reported by various authors.

Author Study	Country	Age (Years)	Sex	Upper Normal Level	% Above Upper Normal Level
Londe (1966)	USA	4-13	M & F		21.0%
Masland et al (1956)	USA	Adolescents	M & F	SBP 140 DBP 90	1.4%
Valkenberg et al (1977)	Holland	5-19	M F	SBP 140 DBP 90	6.5% 2.8%
Lauer et al (1975)	USA	6-9	M & F	SBP 140 DBP 90	0%
Muscatine		14-18		SBP 140 DBP 90	8.9% 4.4%
Heyden et al (1969)	USA	15-25	M & F	SBP 140 and/or DBP	11.0%
			Mean±SD		
Boulton (1979)	South Aust.	8-18	Boys 116.2±16.1	140	8.5%
			Girls 113.3±13		2.5%
			Boys 58.5±11.4	90	10.0%
			Girls 60.3±11.8		8.5%

et al (1976). However, Lauer et al (1977) found that of the 13% of school children in Muscatine, Iowa, who were hypertensive at the first recording, less than 1% had high levels on further examination. Valkenburg et al (1977) found that tracking for BP was variable, and although high levels regressed towards the mean in most children a small percentage of children increased their blood pressure over a four week period.

An underlying polygenic component has been suggested to influence blood pressure levels (Editorial 1978a). Kass et al (1977), found that familial aggregation was well established before the age of two years, and that though it was not demonstratable in the newborn period it became evident soon after the first few weeks of life. This pattern of familial aggregation has also been described for different age groups in English (Holland and Beresford 1975), Canadian (Biron et al 1975), American (Zinner et al 1971; Henneckens et al 1976b) and Polynesian (Beaglehole et al 1975) children. Elevated levels of BP may therefore be present at least as early as teenage, exhibit some degree of tracking, and have a genetic component. The reduced life expectancy of hypertensive individuals makes the early detection of hypertension and control of this disease essential in view of its contribution to the rate of development of atherosclerosis and to CHD risk.

Smoking as a CHD Risk Factor

Cigarette smokers have a greater risk of fatal CHD than non-smokers (Doll and Hill 1964; Hammond 1966; Epstein 1967), with the overall risk being 1.5 to 2.5 times, and for heavy smokers 3.5 times that of the non-smoking population (Roy Coll Phyns, London 1977). This association between cigarette smoking and CHD is independent

of other CHD risk factors (Jenkins et al 1968). However the increased risk mediated by smoking amongst British civil servants with low levels of other risk factors was low (Reid et al 1976), and no association between cigarette smoking and CHD death has been found in men from countries with a low incidence of CHD (Keys 1970). However studies on other comparatively low risk populations including women (Oliver 1974; Mann 1975), American Blacks (Hames et al 1971), and the Japanese (Roy Coll Physicians, London 1977), did show an increased risk of CHD in cigarette smokers.

The rate of CHD in male British doctors aged less than 65 years fell while that in men in England and Wales rose during the same period, being related to many doctors during this period giving up smoking through knowledge of its hazards, compared with the rest of the population (Doll and Peto 1976).

The increase of CHD risk through smoking is greatest in smokers less than twenty five years of age (Doll and Peto 1976), and considerably less for those greater than 65 years old, possibly because of the elimination of those susceptible to the effects of cigarette smoking before this age. Severe and fatal forms of CHD are strongly associated with cigarette smoking as is a higher risk of sudden death (Spain and Brandess 1970). This may be related to nicotine - induced myocardial irritability and subsequent arrythmias.

Angina pectoris has also been causally associated with cigarette smoking (Oran and Sowton 1963), with less angina pectoris being experienced with low-nicotine cigarettes though still present in non-nicotine containing cigarettes (Aronow and Swanson 1969). However, part of this effect may be due to carbon monoxide and not

nicotine (Aronow 1976).

The mechanism by which cigarette smoking increases CHD is probably multifactorial. Although an association between atherosclerosis and cigarette smoking has been reported (Auerbach 1976), it has been disputed because it may have been due to other factors including alcohol consumption, and smoking alone tended to be negatively associated with atheromatous lesions (Lifsic 1976). Carbon monoxide (CO) has been shown to increase the permeability of blood vessels to cholesterol, and changes in the arteries of rabbits analagous to coronary artery disease have been found after inhalation of air containing 0.018% CO (Astrup and Kjeldsen 1974). No effect on the total serum concentrations of cholesterol and triglycerides have been found with cigarette smoking, though changes in a remnant uptake analagous to type III hyperlipoproteinaemia have been proposed as a causal factor in the aetiology of the peripheral vascular disease found in smokers (Topping et al 1977). Kershblaun et al (1963), found that cigarette smoking mobilized free fatty acids (FFA), thereby increasing plasma concentrations, although this may have been due partly to nicotine stimulating the release of catecholamines (Watts 1960). FFA enhance platelets adhesiveness and increase thrombotic tendency, though Levine (1973) found that nicotine itself increased platelet adhesiveness independently of the rise in plasma FFA.

Nicotine is also thought to increase myocardial oxygen demand through increasing heart rate and cardiac output (Herxheimer et al 1967), while decreasing myocardial blood supply, hence increasing the risk of ischaemia. Direct vascular effects have also been implicated for intra-myocardial arterioles have thicker walls in

smokers than non-smokers (Auerbach et al 1971), with an association between the degree of smoking and vessel-wall change. This also could decrease myocardial oxygenation and contribute to ischaemia.

Thus atherosclerosis, thrombosis, and potential borderline myocardial hypoxia have all been implicated in the increased risk of CHD through cigarette smoking.

Smoking may become an established habit in the first decade; 7% of boys and 2-5% of girls aged 10-11 years smoked one or more cigarettes a week with 40% of these English primary school children trying their first cigarette before age nine (Bewley 1978). Of New Zealand adolescents 32% were reported as regular smokers with 15% smoking more than 2 cigarettes a day. Most had begun smoking before secondary school (Beaglehole and Harding 1978). In the National Child development survey in Britain 36% of 16 year olds were self-reported smokers, 9% admitting to being 'heavy'smokers (Pearson and Richardson 1978), and in a sample of Sydney school children studied over a 4 year period 2.3% of boys and 0.5% of girls (average age of 12.7 years) were smoking 20 or more cigarettes a week (Leeder 1977). Four years later 23.6% of boys and 17.6% of girls (average age 15.6 years) were smoking 20 or more cigarettes a week, clearly showing that smoking prevalence increases with age.

Obesity

Although obesity is part of the coronary risk profile, its effect on increasing CHD risk may be mediated through hyperlipidaemia and/or its effect on BP (Leelarthaepin 1974; Pelkonen et al 1977). Excluding hypertensives and diabetics, no independent effect of body build on atherosclerosis was found in a large autopsy series involving several European centres (Sternby 1976). Nonetheless,

the significance of the small additional effect of obesity on CHD risk is considerable (Weinsier et al 1976; Stern 1978), particularly in view of its prevalence in both the adult and childhood population (Wilkinson et al 1977; Court 1977).

Stress

Stress, socio-economic status, and personality factors each play a major role in CHD risk (Zanchetti and Malliani 1974; Jenkins 1976). In the Oslo coronary heart disease study mortality was inversely related to social class, with this being accounted for by differences in prevalence rates of risk factors and to the higher level of physical activity in leisure time in thoseof higher social status (Holme et al 1976). Multivariate analysis has also shown that anxiety and psychosocial problems themselves exert independent effects (Medalie and Goldboort 1976), with the presence of other risk factors augmenting the risk of angina from 14 to 289 per 1000. Furthermore, the correlations which exists between type A behaviour patterns (characterised by striving toward deadlines and achievements, high motivation, and a sense of time urgency), and serum lipid levels, and the presence of a hyperinsulinaemic response to glucose challenge, suggest a causal relationship (Friedman et al 1970).

Physical Activity

Discussion and controversy over the relationship between CHD risk and physical activity began when Morris et al (1953, 1958), showed that London bus conductors had a lower prevalence of CHD than bus drivers though subsequent studies showed that differences in BP and cholesterol alone could have accounted for the effect (Morris et al 1966).

Further epidemiological studies have supported these findings (Shapiro et al 1959; McDonough et al 1965), with evidence that although the probability of sudden cardiac death is greater for men aged 40-59 in sedentary jobs than in strenuous occupations (Kagan 1976b), heavy leisure activity is associated with a lower serum cholesterol, body weight, and BP, and less smoking (Hickey et al 1975). These correlates may depend more on the fact that people who exercise regularly are also more likely to take care about their weight and diet. However in the closely studied population of Busselton, W.A., Cullen and Weeks (1978) reported that only 7% of people purposefully exercised to get short of breath on a regular basis, and that two-thirds exercised less than weekly. There was no difference in CHD risk factor prevalence between those who exercised more or less frequently than four times a week.

Whether physical activity is beneficial in cardiovascular terms through helping to reduce anxiety and stress and by providing an appropriate work load for a stimulated sympathetic nervous system, or by increasing the proportion of high density lipoprotein (Krauss et al 1977), or by a direct effect on coronary vessel size and patency (Currans and White 1961), is in dispute (Editorial 1978b).

It is likely that direct beneficial vascular effects, including resorbtion of atheromatous plaques (Bassler 1978), only occur at extremely high levels of regular exercise such as marathon training, but that less dramatic, though important, effects occur at a lower level of exercise (Kannel 1967; Fox et al 1971).

Prevalence Rates and Clustering of CHD Risk Factors in Children

Although the importance of prevention of atherosclerosis in

early life is recognised (Lloyd and Wolff 1969; Kannel 1976), the relationship between the presence of recognisable risk factors in children and the possible increased risk of CHD cannot be quantified (Elwood 1977). There is nonetheless much data on the extent and prevalence of CHD risk factors in children, with evidence that the increase in CHD mortality over the past twenty five years relates to environmental influences such as obesity, smoking and a high fat diet, which start early in life (Bauer 1977).

Large community screening programmes such as the Muscatine study which involved 4829 children aged from 6 to 18 years (Lauer et al 1975), have described the frequency of risk-associated factors. The incidence of obesity (defined here as >30% overweight) increased with age from 5% in the 6 to 9 year olds to 8% in the teenagers; a systolic blood pressure above the 140 mmHg or a diastolic above 90 mmHg occurred in 8.9% and 12.2% respectively of teenagers, but none of the primary school-aged children; the serum cholesterol level was similar from each group, with 3% having a level greater than 240 mg/dl.

Similar screening studies in Denmark (Strunge and Trostman 1978), and Holland (Kromhout et al 1977; Valkenburg et al 1977) have demonstrated similar prevalence rates for HC, raised BP, and obesity. Concordance of two or more of these factors detected on screening studies were present in 14% of 8 to 12 year old boys, with 46% having one factor, and a history of a relative having had an infarct below 60 years of age in 34% (Wilmore and McNamara 1974). Gillian et al (1977) described concordance for two or more risk factors in 36% of children from a similar age group who underwent a detailed anthropometric study.

In the Bogalusa study Webber et al (1977) used the 75th percentile level as a cut-off point, and found that the concordance rate for a raised cholesterol, BP, and body mass index (BMI) was 3.8% in the White boys, against a predicted rate of 1.2% assuming no biological association. A higher than predicted concordance also occurred for BP and BMI, and cholesterol and BMI, but not for cholesterol and BP.

Positive inter-relationships between cholesterol and body fatness in children have been shown both in large community programmes e.g. Burlington, Vermont (Clarke et al 1970), and in New York (McGandy 1971), as well as in samples of obese children (Court et al, 1974a), and adults (Hollister et al 1967). The correlations were weak but significant, and in particular showed that both moderate HC and hypertriglyceridaemia occurred more frequently in obese children than in slim.

Similarly a positive association has been clearly shown between BP and the degree of body fatness (Kannel et al 1967b; Stine et al 1975), as well as to childhood obesity (Court et al 1974b), with evidence that this relationship may have its origins in early feeding patterns and salt-eating habits (Lauer et al 1976), and be closely related to other parameters of carbohydrate metabolism which themselves may have long term vascular effects (Florey et al 1976).

Tracking of identified risk factors, with those in the higher percentiles remaining in that relative position, has been shown to occur for both cholesterol (McGancy 1971; Godfrey et al 1972), and BP (Zinner et al 1975; Lauer et al 1977), with Clarke et al (1977) describing tracking correlations of 0.38 for systolic and 0.27 for diastolic BP over two and four years. These data therefore

suggest that risk factors remain fixed.

Although the relative importance of familial (genetic) and environmental factors in the aetiology of CHD is impossible to quantitate, CHD nonetheless runs in families (Editorial 1977b).

In a study of risk factors in children of young CHD affected fathers Blumenthal et al (1975) found a higher mean serum cholesterol, but no difference in BP nor triglyceride levels, suggesting that genetic influences on cholesterol levels, short of producing frank HC in children, may mediate CHD risk. How familial CHD risk may be modified by environmental facors was shown in Rissanen and Nikkilä's (1977) family study of Finnish men. Risks of clinical CHD by age 65 to the brother of an affected man varied from 65% in East to 52% in South Finland, compared to 35% and 5% in brothers of control subjects.

The likely importance to a child's future cardiovascular health from the presence of HC, smoking, or a raised BP has been compared to the risk from congenital or rheumatic heart disease in the childhood population (Williams and Wynder 1976). These authors propose that there is sufficient evidence to advocate a lifestyle modification of those at high risk, as has been done in Tuscon (Goldberg et al 1975), and in the three communities programme in California (Farquar et al 1977).

CHAPTER 2

THE SAMPLES

The Children from Birth to Two Years

From November 1st, 1975, 2000 consecutive cord blood samples were collected from the babies born at the Queen Victoria Hospital, Adelaide. Individual details concerning the pre and peri-natal experiences of the babies were not recorded on delivery for logistic reasons, but were retrospectively recorded from the clinical notes of the babies who were subsequently followed up.

Every fourth baby born was selected for the longitudinal prospective study. Each mother was interviewed during her post partum hospital stay; the purpose of the programme was explained to her, and her co-operation was sought. In the event of the mother refusing, the next baby born was chosen for the study. This allowed 500 babies to be enrolled in the study over eight months, from the cohort of 2000 births in that period.

To obtain an initial sample of 500 babies, a total of 600 mothers were interviewed, and 42 mothers were discharged or transferred from hospital before they could be seen. The most common reason for refusal was that the mother (or her husband) was not interested in co-operating. The other common reasons included the baby being offered for adoption, being preterm, or the parents leaving the State. Table 2:1 summarises these reasons in order of frequency.

The "attributes " used in the assessment of socio-economic (s.e.) status were the educational level of the mother, the type of housing the family used, and the parents' address. It was considered that these attributes could be objectively scored and would

TABLE 2:1 Reasons for parents refusing to join the sample, in order of percentage frequency (n = 142).

	n = 42	Percentage
Mother transferred to another hospital or discharged before she could be interviewed		17 %
Husbands refused, mother not interested in cooperating		15
For adoption		14
Baby preterm		13
Parents leaving state		12
Too distant to travel		9
Stillborn		7
Baby sick or died		7
Language problem		4
Major congenital abnormality		2
Parents leaving country		

provide an accurate measurement of the degree of conformity of the sample to that of the whole Adelaide population, without introducing attributes which would be either subject to bias of interpretation e.g. husband's occupation, or invasion of privacy, e.g. income. Tables 2:2 and 2:3 summarise the divisions used, and the sample's profile, for the attributes of maternal education and housing. The parent's address was coded according to the percentage of residents in each suburb with a tertiary education (Cleland and Stimson 1975); rural addresses were given a median rank.

The attributes used to assess ethnic background (Table 2:4) were: country of birth of the parents, the language spoken at home, and the age of the mother on arrival in Australia. The divisions used for the variable "age of arrival of the mother" were arbitrarily taken as under 12 years, over 12 and over 18 years of age. These were chosen as being most likely to have an effect on cultural assimilation, and hence the mother's own child rearing practices.

Analyses of the s.e. attributes of the sample in terms of "address" are shown in Table 2:5 and for "maternal education" and "housing", are shown in Tables 2:2 and 2:3. In this sample, 62.2% had their own house, 29.8% rented, 1% had Housing Trust accommodation, and 6.9% had "other" housing. This compares with the overall Adelaide average percentages for owner-occupancy of 69.9% renting 16.9%, Housing Trust 9.8% and "other" 3%. The shift on the sample profile for this attribute through the two years is shown in Table 2:3, with the average percentage values for Adelaide for comparison.

TABLE 2:2 Educational level of mother; the percentage frequency of the level of educational attainment of the mother. Values for the sample for three (391), and six months (325), those attending at three months only (66), and one year (289), two years (198), those who attended at one but not two years, and the Adelaide average for achieving tertiary (degree) and intermediate/leaving level.

	3 months	Attenders at 3 months only	6 months	1 years	2 years	Attended at 1 but not 2 years	Adelaide average
Minimum school leaving age	51	61.4	49.1	47.2	45.4	51.7	
Intermediate/leaving	24	18.6	25.5	27.3	27.6	24.7	28.5
Matriculation	7.7	8.6	7.5	7.3	9.2	3.4	
Tertiary (non-university)	8.7	5.7	9.3	9.1	9.7	10.1	(
Tertiary (university)	8.0	5.7	8.7	9.1	8.2	10.0	(5.25

TABLE 2:3 The percentage frequency of the type of housing used. Values for the sample for three (391), six (325), and 12 months (289), those attending at three months only (66), and the average values for Adelaide.

Attenders at (months):	3	6	12	3 months only	Adelaide average
Owner-occupier	62.2	65.1	68.1	49.3	69.6
Rented	29.8	28.4	25.3	36.2	16.9
Housing Trust	1.0	0.9	1.9	1.4	9.8
Other	6.9	3.6	5.6	13.0	3.0

TABLE 2:4 Ethnic attributes of sample

A. Country of birth of mother/father:

Australia	Italy
United Kingdom	Greece
N. America	Yugoslavia
New Zealand	Other

B. Language spoken at home:

English	Yugoslav
Italian	Other
Greek	

C. Age of arrival of mother (if migrant): Less than 12 years

12 - 18 years

Over 18 years

TABLE 2:5 The percentage frequency of attenders from three months to two years according to address, ranked from 1 to 9, and for those who attended at one but not at two years.

Address rank	3 months	6 months	1 year	2 years	Attended at one but not two years
1	9.3	10.2	11.0	10.5	17.3
2	7.9	8.3	8.0	9.9	8.0
3	6.1	6.2	4.8	5.8	6.7
4	11.8	11.1	10.8	14.0	8.0
5	17.6	18.2	19.0	22.0	22.7
6	4.9	4.4	3.8	4.7	5.3
7	9.2	8.6	9.0	9.3	13.3
8	13.0	12.0	12.5	14.5	14.7
9	20.2	21.0	21.1	9.3	4.0

The percentage of mothers with university education was 8%, with tertiary (non-university) 8.7%, up to matriculation level 7.7%, to intermediate or leaving level 24%, and to the minimum school leaving age 51%. This compares with the average for Adelaide of 5.25% with tertiary education and 28.5% to leaving level. The change through the year in the profile of the sample's education attribute was towards those with tertiary education (+1.4%) and intermediate level (+3.3%), with a slight decline in those with matriculation level (-0.3%), and minimum school leaving attainment (-3.8%). A minor shift at the expense of those for minimum school leaving occurred, with an increase for those in the next two categories; Table 2:2.

Table 2:5 shows the percentage distribution for the parents' address ranked in codes from 1 to >9, from three months to two years, with the percentage who attended at one but not two years. A slight shift of the sample towards a higher s.e. status through the first year occurred with disproportionately fewer mothers failing to re-attend at six and twelve months from suburbs ranked 1 and 2 at the expense of middle-ranked addresses, though the percentage of mothers attending from suburbs ranked 8 and 9 changed little. Dividing the address rank into quintiles, the difference in percentage from three months to the one year attendance is shown in Table 2:6.

Seventy percent of the mothers were born in Australia (Adelaide average 71.9%), 11.8% in the U.K. (average 3.4%), and Greece 4.4% (average 1.4%). Table 2:7 shows the change in sample and Table 2:8 shows the percentage frequency for the language used at home and its changes through the two years.

TABLE 2:6 Percentage difference in attendance from 3 to 12 months

Quintile of address rank	Percentage difference
1	+1.9
2	-2.3
3	+0.3
4	+0.6
5	+0.9

TABLE 2:7 Country of origin of mother. The percentage of the sample attending at three (391), six (325), and 12 months (289), those who attended at three months only (66), and the Adelaide average percentage.

	3 months	6 months	12 months	Attenders at 3 months only	Adelaide average
Australia	70	71.9	72.2	61.4	71.9
U.K.	11.8	12	12.2	11.4	14.8
Italy	3.8	4	3.8	2.9	3.4
Greece	4.4	3.4	3.1	8.6	1.4
Yugoslavia	0.8	0.3	0.3	2.9	0.7
Other	9.2	8.3	8.3	12.8	7.7

TABLE 2:8 Language used at home. The percentage of the sample attending at three (391) and six months (326), and one year (289), and those who attended at three months only (66).

Attenders at:	3 months	6 months	1 year	3 months only	2 year	Attended at one but not two years
English	90.7	92.0	93.7	81.4	93.9	99.2
Italian	3.3	3.1	2.6	4.3	4.0	-
Greek	4.6	3.4	3.1	10.0	1.5	6.8
Yugoslav	0.3	0.6	0.3	1.5	-	-
Other	0.1	-	-	2.9	0.5	-

Table 2:9 summarises the change in sample profile for the attribute "age of arrival of mother". Those mothers who had migrated at primary school age (<12 years) and up to 18 years were increasingly represented through the first year.

The change in the sample's profile for these two attributes received a substantial contribution from non-English speaking people, with 18.6% of those failing to attend at the six month visit speaking another language. The change in profile of the country of origin of the mother shows that Greeks and "others" (non specified minority groups) were disproportionately represented amongst non-attenders after three months. However, the small number (4 people) in the "other" category prevents weight being ascribed to this observation.

The children were seen at three, six, twelve months, and at two years of age in a special clinic in the Adelaide Children's Hospital. Before the mother's first visit, when the baby was three months old, a letter and appointment card were sent. In the event of her failing to attend, a further letter was sent and an attempt was made to make contact by telephone, or through a visit by a Mothers and Babies' Health Association Sister. This latter arrangement was used only for the first two months of the study because of the logistic problems involved. Of the 500 mothers, 391 were seen at the three month visit (78.9%), leaving 109 mothers who failed to attend. Information about the reasons for non-attendance was gained through contact made to the mother or her relatives, although in 24.8% (n=27) this was unsuccessful. A further 32 mothers were visited, and the stated reasons for non-attendance have been included in Table 2:10, in which is summarised

TABLE 2:9 Age of arrival of the mother in Australia. The percentage frequency of migrant mothers (N = 113).

Attenders at (months):	3	6	12
Under 12 years	49.6	50.6	51.3
12 to 18 years	12.4	13.8	14.1
Over 18 years	38.1	35.6	34.6

TABLE 2:10 Reasons for failing to attend the first visit, as a percentage in order of frquency (n = 109).

	Percentages
Too far or transport difficulties	25.6
Not known	24.8
Single parent	15.6
Not interested	10.1
No forwarding address	9.2
Moving interstate	4.6
Language problem	4.6
Baby died or adopted	2.8
Other - deaf and dumb mother	0.9
baby sick	0.9
mother worked	0.9

the percentage frequency for events associated with non-attendance. 14.8% (n=17) of this group of non-attenders were single girls, compared with 7% (n=15) of the mothers who attended the three month clinic; only a slight skewing towards suburbs of lower s.e. status occurred in the non-attenders.

325 babies (65%) were seen at the second visit at six months, 289 babies (57.8%) at twelve months, and 198 children (40%) were seen at two years.

Before the two year visit a questionnaire was sent out concerning details of the families' experience of coronary heart disease (angina or myocardial infarct), hypercholesterolaemia, hypertension, stroke, peripheral vascular disease, and diabetes.

An infarct or angina under 65 years of age had occurred in:

2 fathers	1 mother
48 paternal grandfathers	34 maternal grandfathers
17 paternal grandmothers	20 maternal grandmothers
35 paternal great grandfathers	35 maternal great grandfathers
18 paternal great grandmothers	35 maternal great grandmothers

These data were used in the analysis described in Chapters 7 and 11.

The Sample of Four-Year Olds

This comprised 244 boys aged from 3 years 8 months to 5 years 3 months, and 242 girls aged 3 years 6 months to 5 years 2 months (Figure 1). They were all in good health, and were seen as part of a comprehensive pre-school health screening programme.

The sample was representative of the Adelaide population in terms of parental occupation (Table 2:11), the country of birth of the parents (Table 2:12), with a slight over-representation of parents born in Britain rather than in Australia.

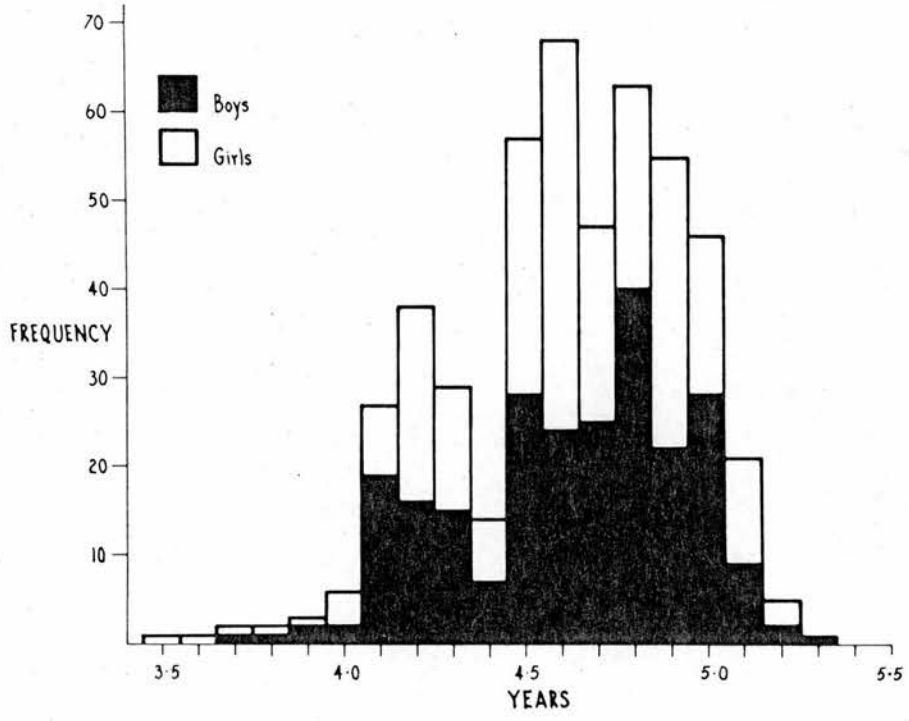

Fig 1. The age and sex distribution of the four-year olds' sample (244 boys, 242 girls).

TABLE 2:11 Occupation of parents (percentage frequency).

	Prof./Exeuc.	Business	Manual	Casual/Unemp.	Home Duties	Other	Number
Mother	2	4	6	9	76	2	417
Father	18	24	49	2	-	8	410
Adelaide average for men	19	33.5	48				

TABLE 2:12 Country of origin of parents, for four year old sample (percentage frequency).

	Aust.	U.K.	N.Z.	Italy	Greece	Yugoslavia	Other	Number
Mother	64	20	1	5	2	1	7	417
Father	59	17	-	8	2	-	13	413
Adelaide average	72	14.8	-	3.4	1.4	0.7	7.7	

The School-age Sample

The children studied were selected by random number technique from each 12 month age group from 8 to 18 years. The primary and secondary schools used were both located in a median ranked suburb for socio-economic status. 117 girls and 118 boys, whose age ranged from 8.2 to 17.9 years, took part (Figure 2). Their S.E. status was defined from the father's occupation using the Congalton criteria (Congalton 1969) (Figure 3). The country of origin of the children's parents also conformed to the overall Adelaide average (Table 2:13).

The children were grouped into seven age categories, which were arbitrarily chosen to allow satisfactory numbers to be included in each group, with greater separation into smaller groups at one year intervals for those children going through pubertal changes from 11.5 to 14.5 years. The mean ages and numbers in each category are summarised in Table 2:14. Any advantage gained from dividing the groups into narrower age ranges would have been lost due to the greater within-group variance of the smaller subsample size.

Before the study a questionnaire concerning the families' experience of CHD was completed by all the children and the details were enlarged upon as necessary during an interview with each child. An infarct or angina occurring under 65 years of age had occurred in:

7 fathers	1 mother
52 paternal grandfathers	36 maternal grandfathers
18 paternal grandmothers	26 maternal grandmothers

These data were used in the analyses described in Chapter 12.

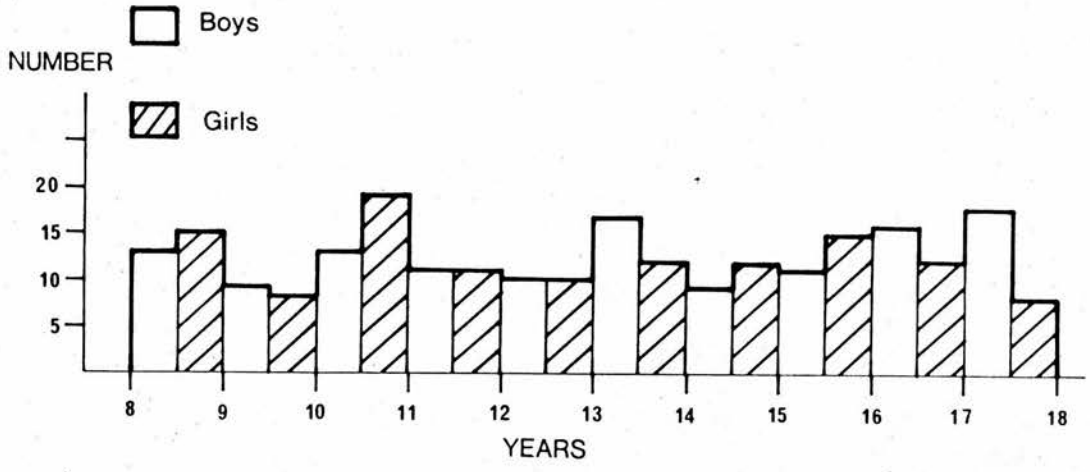

Fig 2. The distribution of the schools sample by age and sex (118 boys, 117 girls).

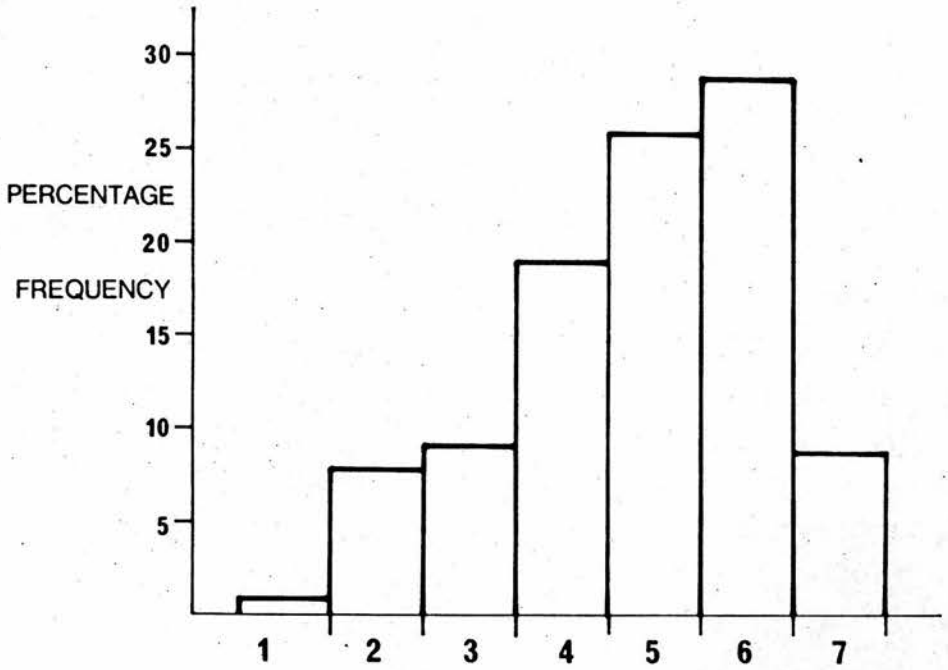

Fig 3. The frequency distribution of socio-economic status of the schools sample based on occupation of father (Congalton rating).

TABLE 2:13 Country of origin of parents, for school sample (percentage frequency).

Country	Mother	Father	Adelaide average
Australia	71.4	73.2	72.0
U.K.	12.1	9.9	14.8
Italy	1.3	1.7	3.4
Greece	3.7	3.7	1.4
Yugoslavia	0.8	0.8	0.7
Other (including N. European)	10.7	10.7	7.7

TABLE 2:14 The ages for each age category in the school sample.

Age Category (years)		n	Age (years) mean	± SEM
<9.5	Boys	(17)	8.72	0.08
	Girls	(16)	8.81	0.11
9.5 - 11.4	Boys	(26)	10.49	0.12
	Girls	(26)	10.57	0.1
11.5 - 12.4	Boys	(8)	11.96	0.11
	Girls	(15)	11.96	0.08
12.5 - 13.4	Boys	(18)	13.05	0.08
	Girls	(7)	13.03	0.13
13.5 - 14.4	Boys	(12)	13.88	0.09
	Girls	(15)	13.96	0.07
14.5 - 16.4	Boys	(26)	15.64	0.13
	Girls	(28)	15.56	0.11
>16.5	Boys	(15)	17.08	0.11
	Girls	(12)	17.16	0.11

Statistical Analyses

All the data elements were recorded on computer file and analysed using the standard statistical techniques incorporated in the Statistical package for the Social Sciences (Nie et al 1975).

Commentary on Sampling Methodology

For valid conclusions to be drawn about the prevalence, normal levels and the inter-relationship of health-related factors of biochemical measurements, the sample concerned must be representative of the population under study. However, during longitudinal studies the subjects act as their own controls, so that patterns of change may be observed without reference to the representativeness of the (perhaps) shrinking sample. In the first sample the participating babies and their mothers were a representative cross-section of their community. The Queen Victoria Hospital was chosen for obtaining the sample because it is the largest delivery unit in Adelaide, serving as a referral centre for obstetric patients and providing routine care to normal mothers. Because of the intrinsically random nature of birth order in such a unit, the systematic selection of every fourth child born was considered not to introduce bias into the sampling method. Though the main reasons for non-selection into the sample (mother not interested, baby for adoption, and the baby being preterm) may each have contributed a poorly-definable bias into the sample, the similarity of the sample's socio-economic status to average figures for Adelaide (Cleland and Stimson 1975), suggests that this bias was not significant, and that it did not change through the two year study period.

The second source of potential bias concerns the 21.8% of

mothers who were not seen at the first visit. Of these, one half either had transport difficulties, or lived too far distant or no reason was determined. Of the remainder, the majority had reasons which were sociologically related, e.g. single parent, not interested, no forwarding address, or language difficulties, etc.

Further potential for bias occurred with the decline in attendance to 65% at six months and 57.8% at one year, so that the data for prevalence at one year could have been biased.

Several of the objectively assessed attributes of the socio-economic (educational level of the mother, housing) and ethnic (country of origin of mother) background of this sample were compared with data from the analysis of the 1971 census (Cleland and Stimson 1975), and were found not to be significantly different. However, as the average values for Adelaide from the 1971 census figures apply to all ages of both sexes, then deviations for some attributes, e.g. home ownership, could be expected for a sample of young women.

Analysis of the amount of change for each attribute for the whole sample population through the two years showed a slight trend only towards higher socio-economic status. The percentage for owner-occupancy of housing rose steadily to 68.1%, nearly reaching the Adelaide average value of 69.6%. The proportion of mothers with both intermediate and tertiary educational level rose slightly at the expense of those with a minimum school leaving age attainment. For ethnic background, a slight shift in the population was noted towards English-speakers, with the proportion of Australian and English born mothers increasing by 2.2% and 0.4% respectively at one year. This was at the expense of Italian and Greek speaking

mothers, although the contribution from those born in Italy remained constant. The percentage of those who migrated here at under 12 years of age, and from 12 to 18 years of age shows an increase through the first year at the expense of those mothers who arrived after they were 18 years old. These figures suggest that the fall-off in attendance received a large contribution from non-English speaking migrant mothers (in particular Greek) who had received no schooling in Australia.

The profile of the sample's address ranked according to social class, showed a small change by one year with the increased representation of mothers from higher ranked addresses coming at the expense of the upper-middle rank, with the contribution from the lower three ranks remaining static. By two years the drop-out rate had come largely from the lowest ranked address, though there was no overall bias, due to under-representation of low s.e. status mothers.

The sample of four-year-olds was sufficiently large for valid conclusions to be drawn from its measurements. A small proportion of the children were followed for approximately one year to provide data on tracking at that age (see Chapter 12).

The schools sample was carefully selected by random number technique to provide accurate cross-sectional data from children going through puberty. Because of the detail of the studies in which they were involved (the subject of other endocrinological, nutritional, and anthropometric reports) the sample size was for logistic reasons kept below 250 children.

Although there are inherent potential methodological and statistical errors when grouping a sample in which the variable

to be measured increase with age (Healey 1962), resulting in a larger standard deviation than if all the children in the sample had been exactly 12.0 years instead of from, say, 11.5 to 12.4 years, the rate of change of the variables under consideration was considered to be sufficiently show that an appreciable error would not have been introduced.

In conclusion, the samples selected were representative of the Adelaide Child population and provided adequate numbers on which to base assumptions from analyses of their anthropometric and biochemical measurements.

CHAPTER 3

ANTHROPOMETRIC AND NUTRITIONAL STUDIES

a. Height and body mass measurements

During infancy body mass measurements were performed with the child supine on a Seca beam-balance baby scales to 10 gm, and at two years to 100 gm with the child standing using beam-balance scales. The length was measured with the child lying supine to 1 mm on a Holtain baby measuring table.

The four-year-olds and school samples were measured on a Seca beam-balance scale to 100 gm and their heights were measured to 1 mm with a Holtain Stadiometer using a standard technique (Tanner et al, 1965).

The mean values for the heights and body masses of each group conformed to Australian standards (NH & MRC, 1975);Figures 1 to 8 and Table 3:1.

b. The skinfold thickness

This was measured at the left mid-biceps, triceps, subscapular, and suprailiac sites with the child sitting on his/her mother's lap, or standing, using Harpenden calipers (Tanner and Whitehouse, 1975). From the sum of the four skinfold thickness measurements (SFT) the value of the percentage of body weight as fat (BWF) was calculated, using a method based on densiometric studies (Brook 1971). From the BWF the lean body mass (LBM) of each child was also computed. The values for SFT at each age are presented in Table 3:2 and as percentiles in Figures 9 and 10.

c. Blood pressure

The blood pressure was recorded on the one-year-olds using a standard mercury sphymamonometer, and on the two-year-olds using an Arteriosonde doppler apparatus. The four-year-olds'BP was

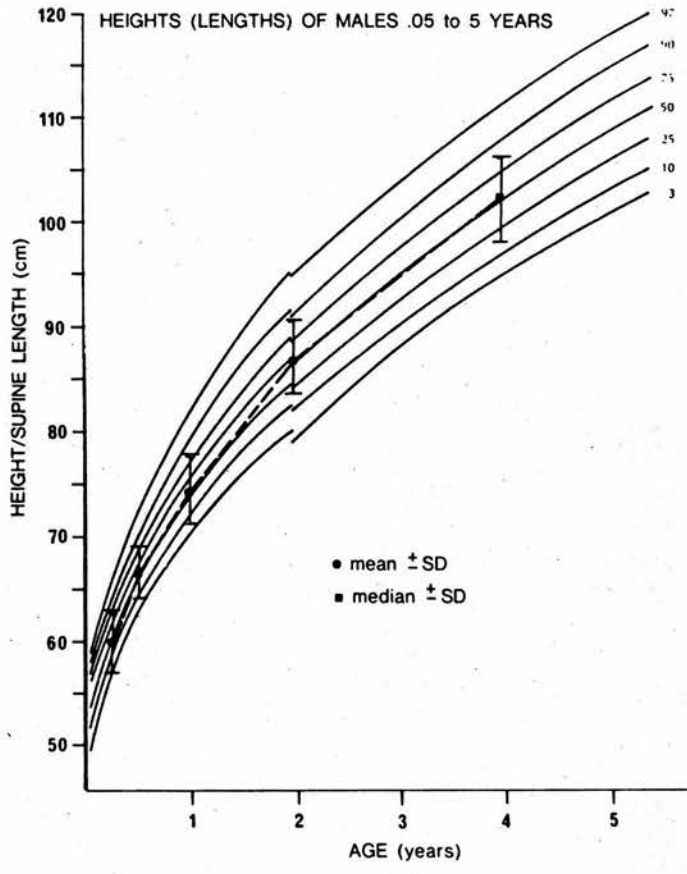

Fig 1. Heights of boys in sample from three months to four years

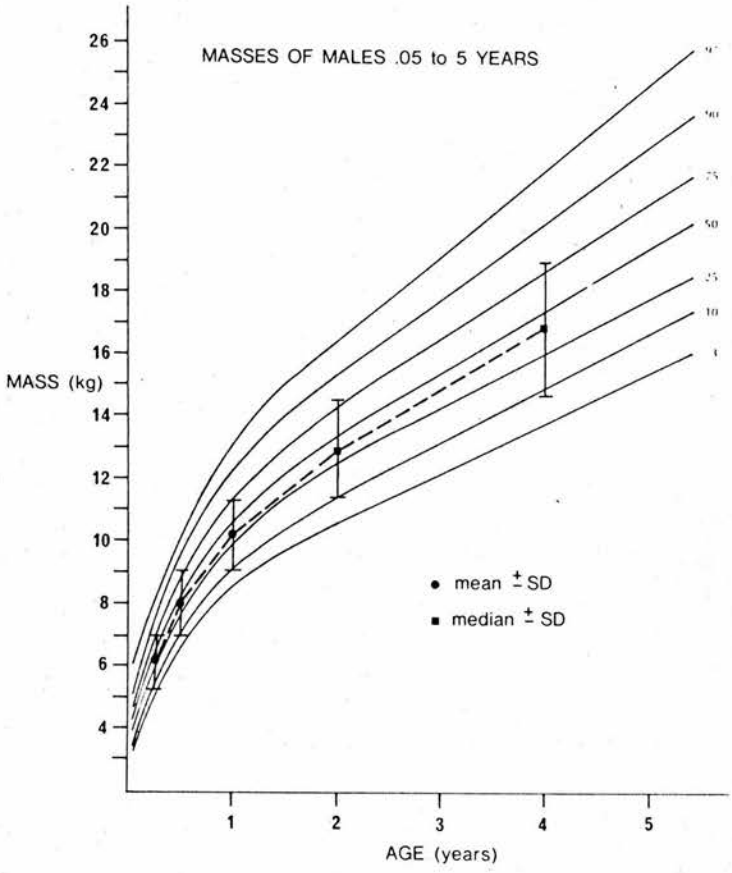

Fig 2. Body masses of boys in sample from three months to four years.

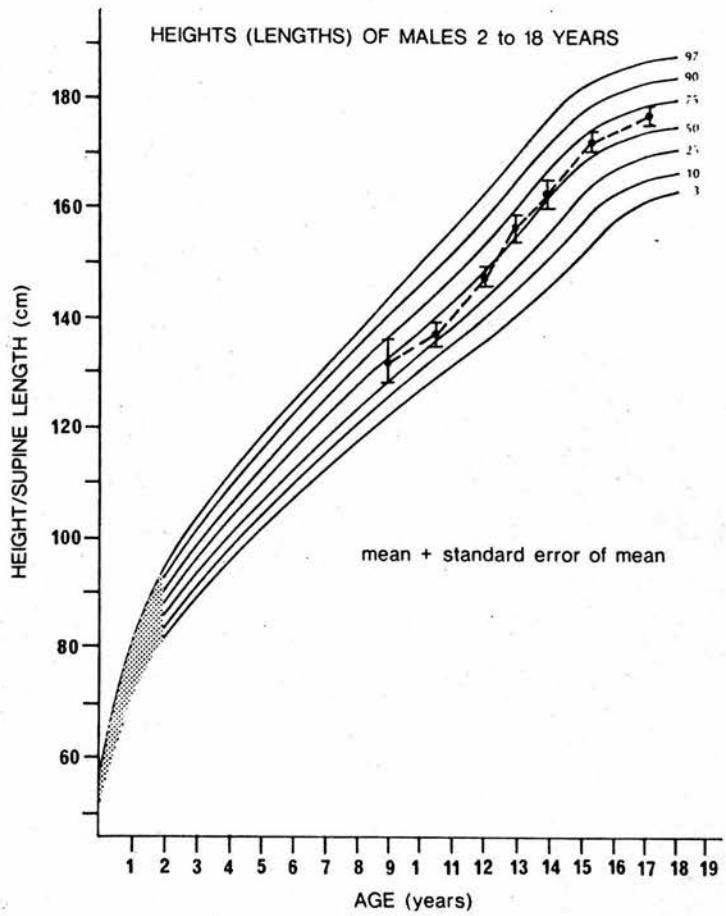

Fig 3. Heights of boys in schools sample

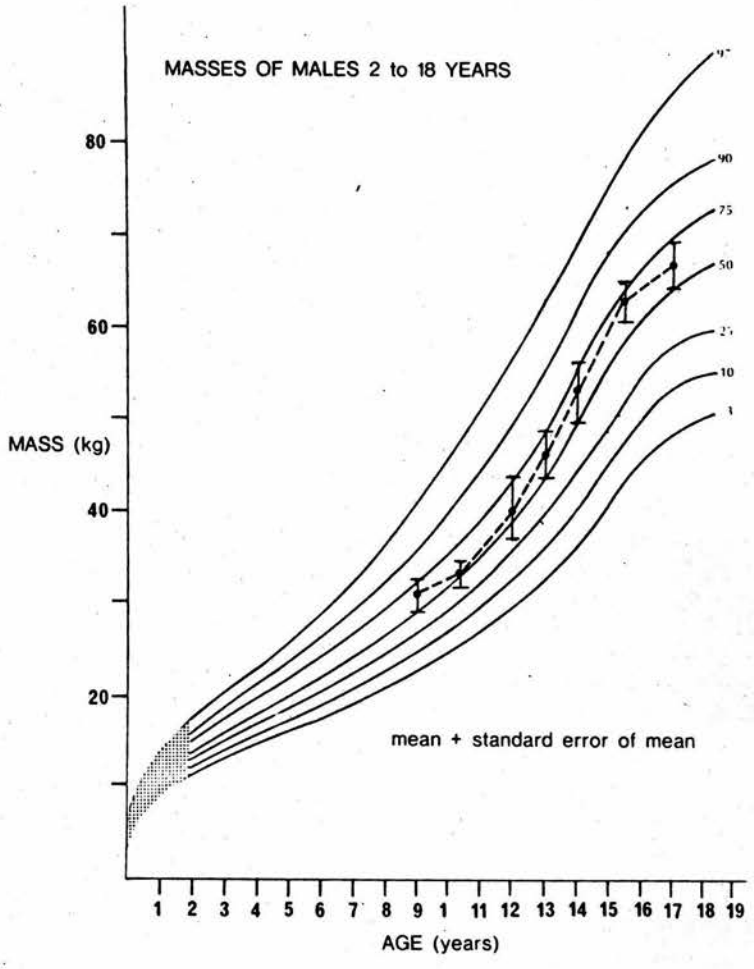

Fig 4. Body masses of boys in schools sample

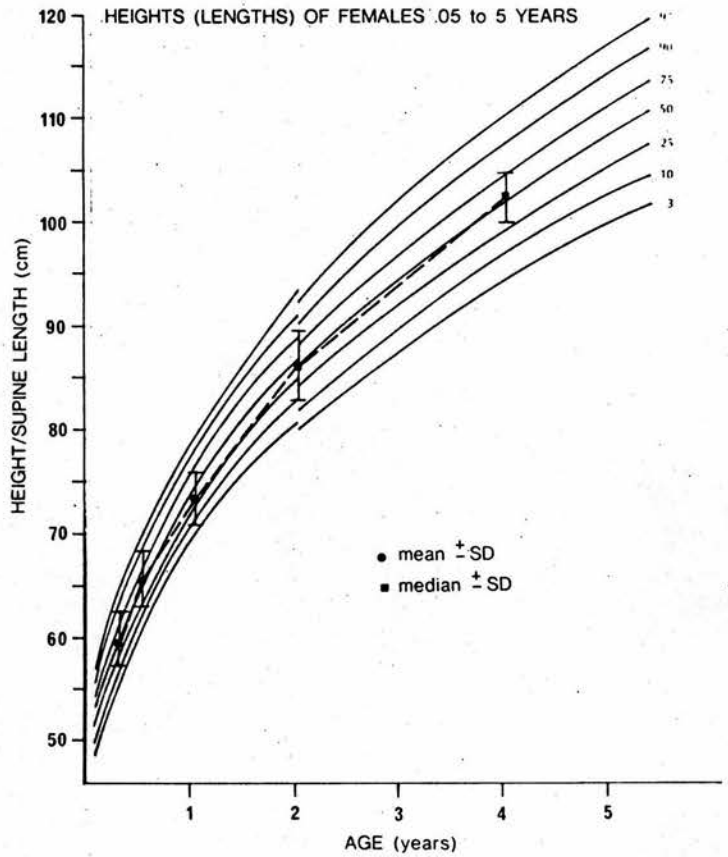

Fig 5. Heights of girls in sample from three months to four years

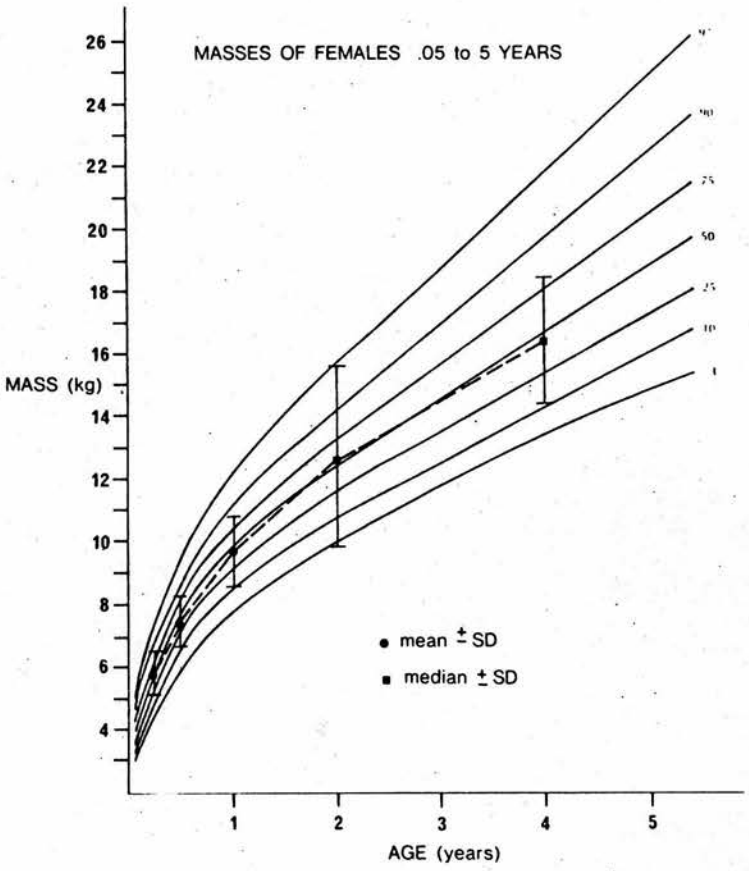

Fig 6. Body masses of girls in sample from three months to four years

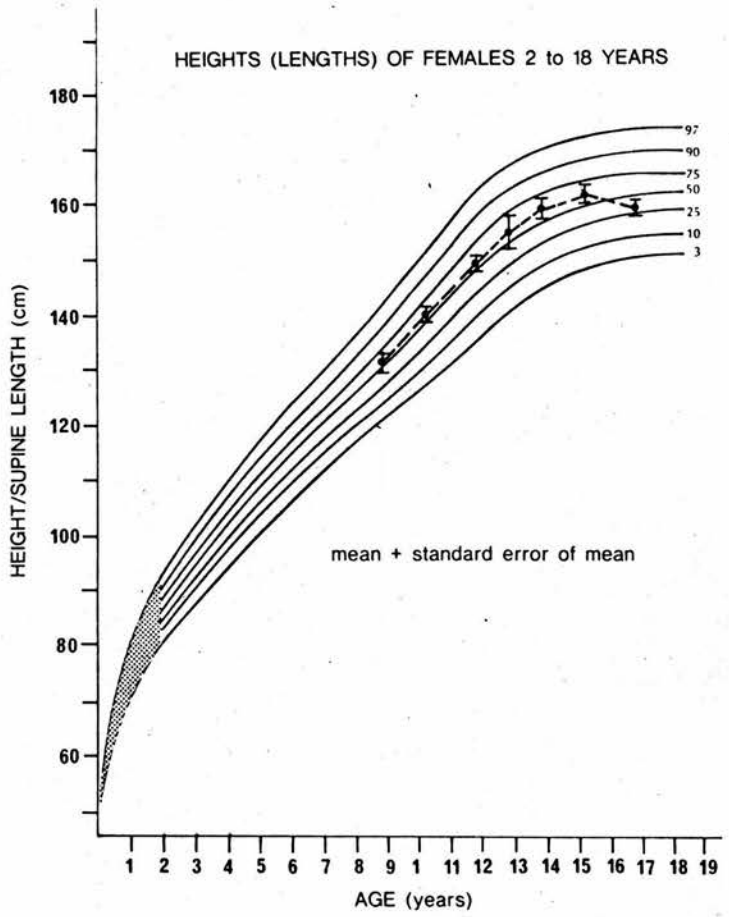

Fig 7. Heights of girls in schools sample

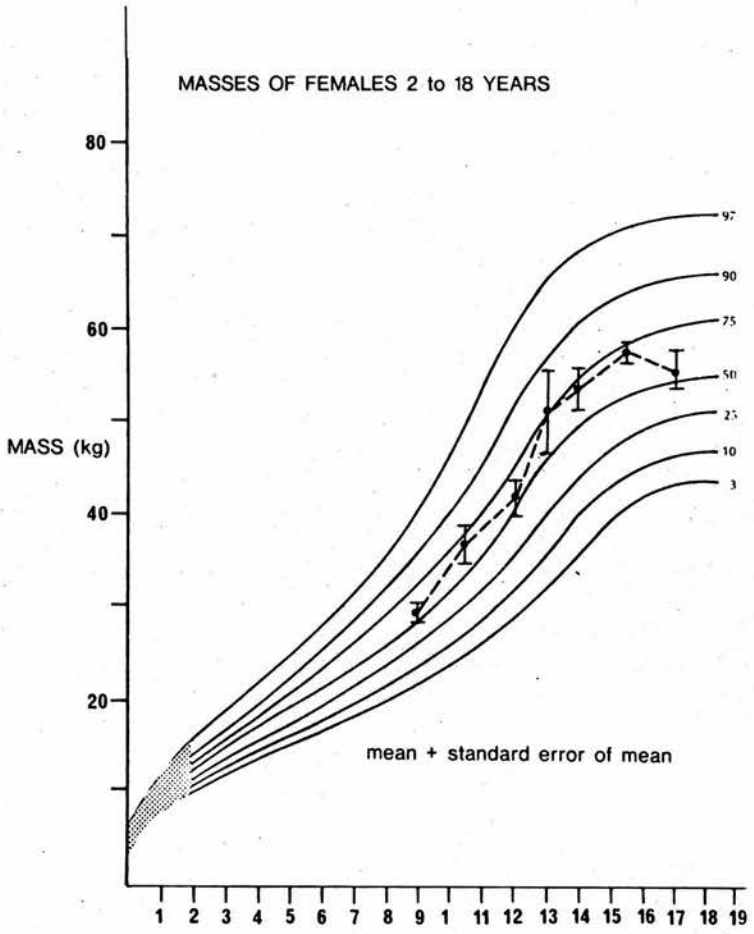

Fig 8. Body masses of girls in schools sample

TABLE 3:1 Heights and body mass measurements of the children from three months to two years. (m = male, f = female).

Age (months)		n	Height (cm) mean	S.D.	Mass (kg) mean	S.D.
3	m	185	61.03	2.61	6.07	0.77
	f	192	59.74	2.39	5.62	0.68
6	m	163	67.32	2.59	7.89	0.95
	f	159	65.66	2.24	7.31	0.84
12	m	144	75.35	2.99	10.51	1.23
	f	142	73.58	2.66	9.57	1.13
24	m	102	87.79	3.49	12.94	1.67
	f	96	86.67	2.99	12.65	2.92

TABLE 3:2 The values in mm for the sum of the four skinfold thickness measurements taken at each age.

		Mean	S.D.	Range	n
3 months	Boys	33.02	6.04	16-54	185
	Girls	32.64	6.22	17-50	192
6 months	Boys	34.95	6.08	22-54	163
	Girls	34.98	6.40	18-52	159
1 year	Boys	33.09	7.23	19-53	143
	Girls	34.59	7.82	20-59	140
2 years	Boys	31.66	6.38	19.5-52.2	102
	Girls	33.70	7.34	20.8-55.6	93
4 years	Boys	24.80	7.17	14.0-60.0	239
	Girls	27.81	8.70	14.0-73.0	233
9 years	Boys	35.80	20.29	19-85	16
	Girls	44.82	24.66	24-117.6	17
10.5 years	Boys	30.63	13.80	17.6-60.8	23
	Girls	58.88	35.92	21.2-137.8	22
12 years	Boys	49.45	29.83	17.4-95.6	8
	Girls	51.87	25.24	21-99	15
13 years	Boys	41.4	14.83	17-64.8	16
	Girls	71.29	25.91	40.2-115.2	7
14 years	Boys	47.1	17.35	24-83	12
	Girls	61.55	29.64	29-138.4	15
15.5 years	Boys	45.50	25.39	242-120.6	25
	Girls	68.70	24.51	27.2-130.6	27
17 years	Boys	45.23	7.22	24.6-47	15
	Girls	65.63	22.73	36.4-117	12

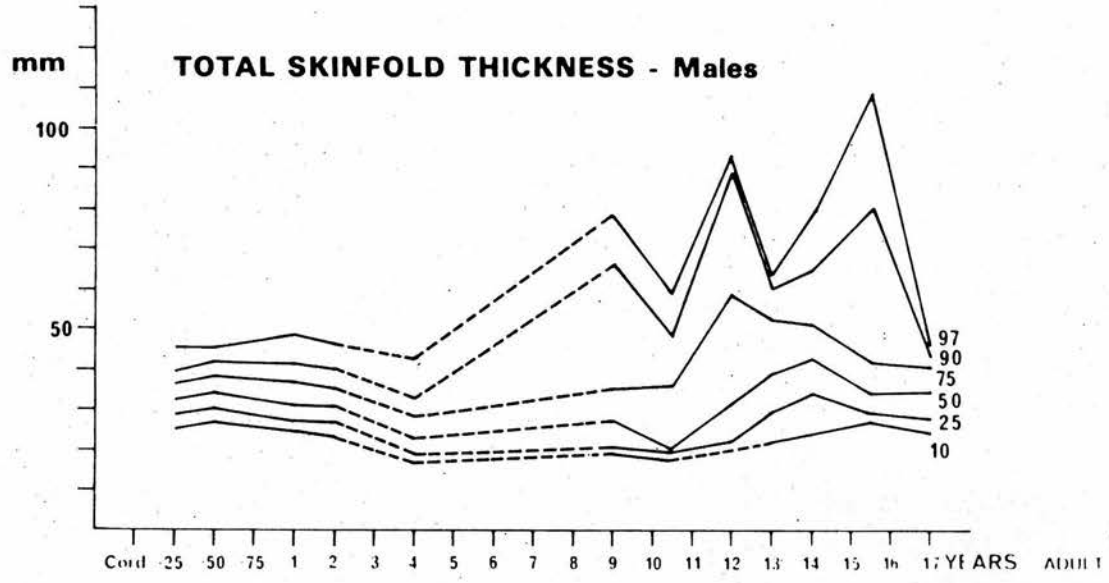

Fig 9. Percentile values for the sum of four skinfold thicknesses (mm) in the boys from three months to 17 years of age.

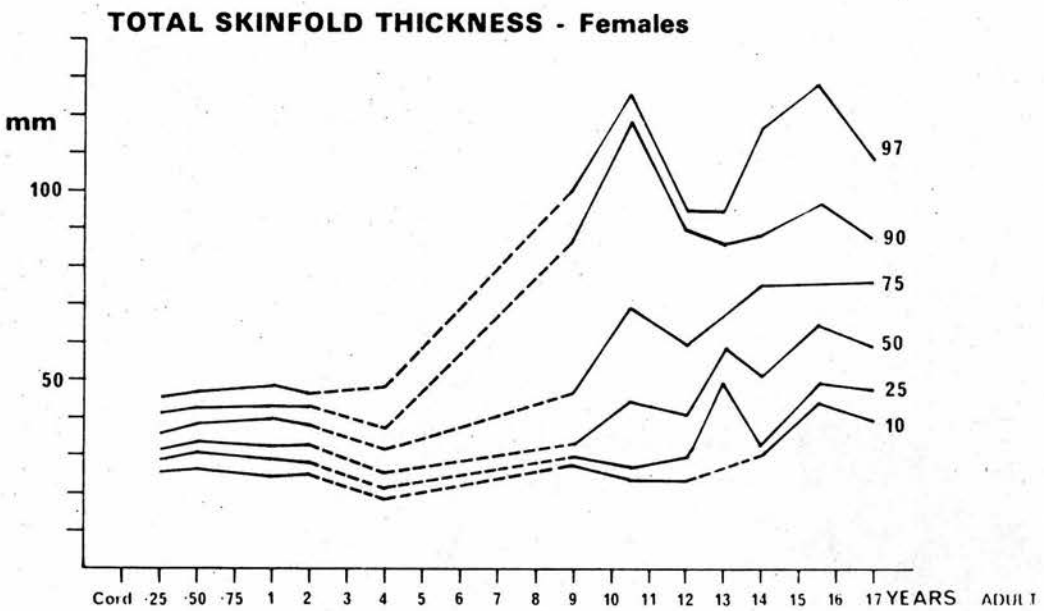

Fig 10. Percentile values for the sum of the four skinfold thicknesses (mm) in girls from three months to 17 years of age.

recorded with the child sitting, using a mercury sphygomanometer with a 10 cm cuff. The random zero technique was not used. The diastolic pressure was read at the 4th Korokow phase. The school age children's BP was measured with the child lying after a five minute rest. The largest comfortable cuff size was used for each child (22.5 x 12 cm) and the diastolic pressure was recorded at the 4th phase. The values for the BP at each age are shown in Table 3:3.

d. The pubertal status of the children in schools' sample was assessed using Tanner's (1962) criteria, with the testicular volume being measure with a Pradi orchidometer and expressed as a mean value, and the pubic hair, breast, and genital development being graded from 1 to 5.

The changes in pubertal status according to chronological and bone age are shown in Figure 11, and the inter-relationships between the stages of development of pubic hair and testicular volume (boys) and breast development (girls) are shown in Figures 12 and 13.

e. The bone age was measured on the school children from a wrist radiograph using the TW2 method (Tanner et al 1972). Figures 14 and 15 shows this expressed against chronological age.

f. The school children underwent a standard physical work capacity (PWC) (Van Dobëln 1954), which was performed on a mechanically braked Monark bicycle ergometer. The children used the senior or junior model according to size and age, and a standard calibration procedure was used, (Wahlund 1948). Three successive three-minute workload tests were used, with one-minute rest periods between each test. Trained physical education staff adjusted the workloads to

TABLE 3:3 The values for blood pressure for each age group.

	Age (years)	(n)	SYSTOLIC				DIASTOLIC			
			mean	SD	Range	95th percentile	mean	SD	Range	95th percentile
Boys	1	137	93	10	60-120	106	54	8	30-75	65
Girls		132	89	11	60-120	105	52	8	30-70	65
Boys	2	96	86	15	58-122	110	47	10	15-70	59
Girls		84	85	12	65-130	104	47	10	20-70	60
Combined	4	452	91	10	60-140	105	59	9	10-90	70
Boys	<12.5	49	105	10	86-132	122	56	9	40-78	70
Girls	<11.5	39	102	10	88-130	123	54	11	36-72	68
Boys	12.5-14.5	28	122	17	96-170	152	57	11	40-92	80
Girls	11.5-14.5	34	115	9	100-142	130	59	9	36-82	72
Boys	>14.5	40	126	13	106-160	148	62	13	40-98	82
Girls		39	122	11	102-150	138	68	11	50-106	82

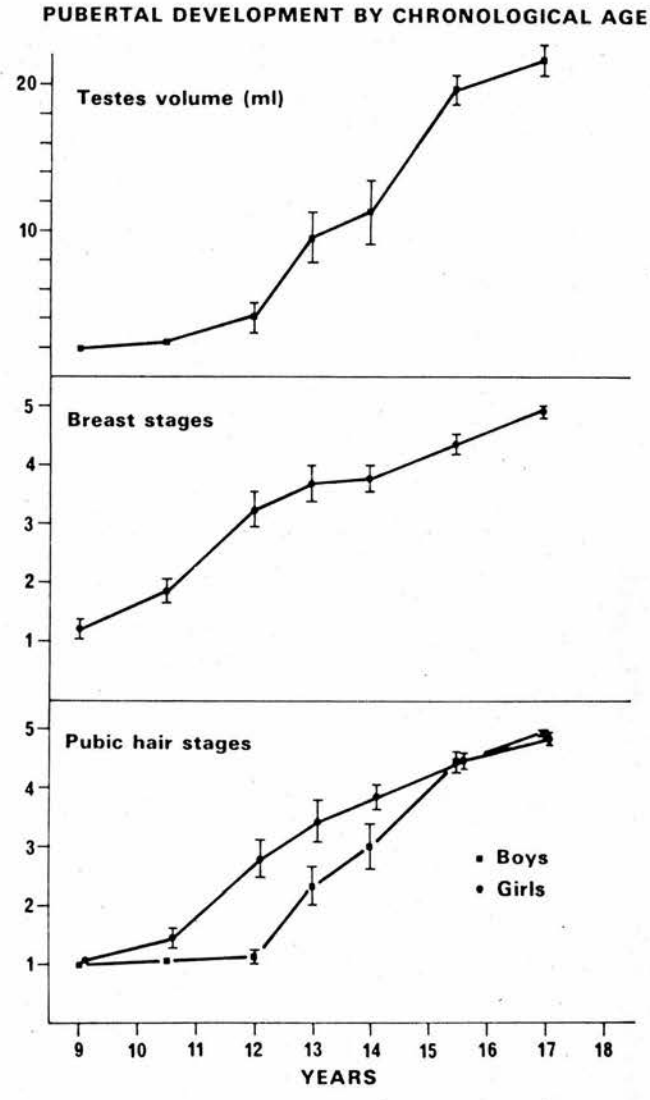

Fig 11. The values recorded for mean testicular volume (± S.E.M.), girls' breast development (Stages 1 to 5, ± S.E.M.), and pubic hair development for boys and girls (Stages 1 to 5, ± S.E.M.) according to age.

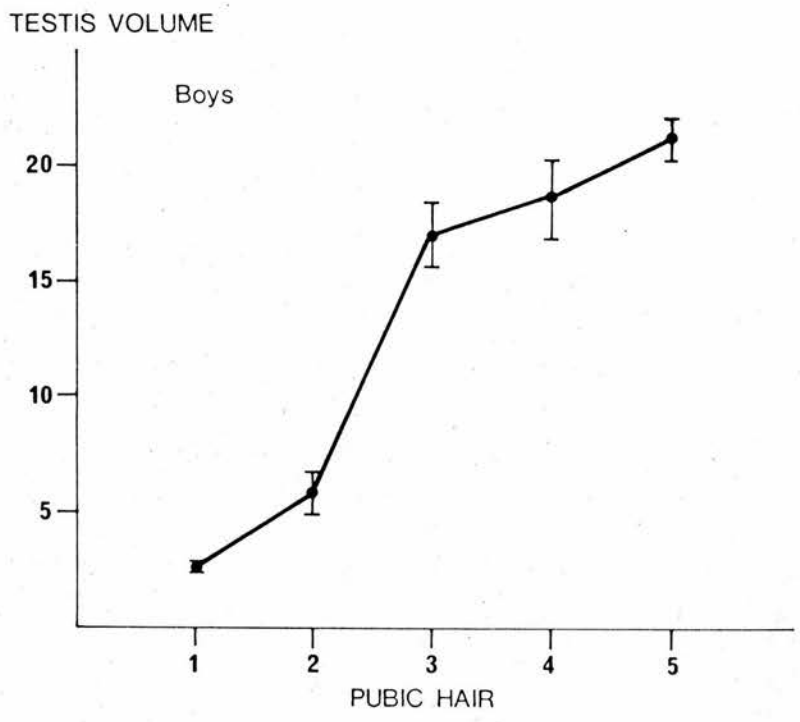

Fig 12. Mean testicular volume (± SEM) according to mean age for pubic hair development Stages 1 to 5.

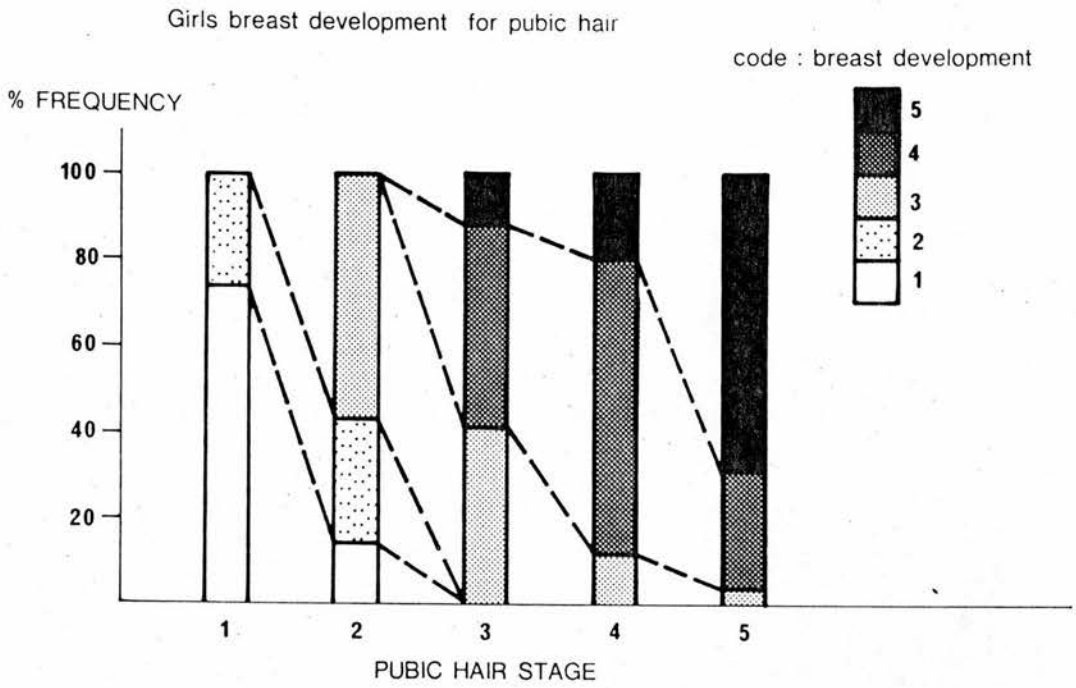

Fig 13. Frequency distribution of stages of girls' breast development according to mean age for pubic hair development Stages 1 to 5.

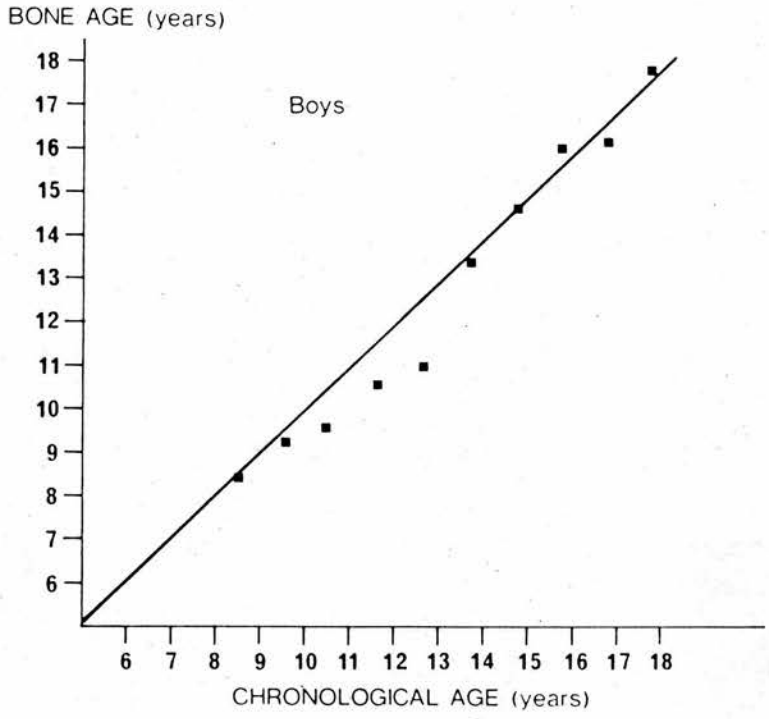

Fig 14. Values for bone age plotted against chronological age for boys.

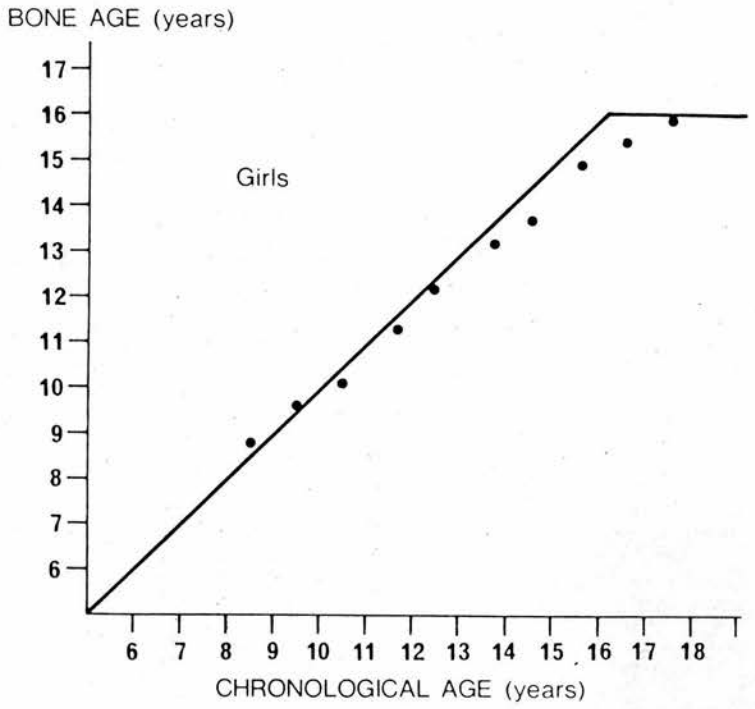

Fig 15. Values for bone age plotted against chronological age for girls.

cause the children to achieve heart rates of 100 to 180 per minute. The rates were measure by auscultation over the last fifteen seconds of the second and third minutes' testing. A fourth workload was used on some of the older boys who had not reached a heart rate of 150 per minute by the third test. Tests were stopped if the child became tired and this occurred even when the heart rate was below 150 per minute in several slightly built children.

The PWC at a heart rate of 150 per minute (PWC 150) could be calculated directly from each child's performance, and did not require the extrapolation that would have been required for deriving the values for PWC 170. Adjusting the PWC 150 values to body mass gave the measurement in kiloponds per metre per minute per Kg body weight. The results for the PWC 150 for each age group are shown in Figure 16.

g. Nutritional studies

Before each visit during infancy the mother was asked to complete a dietary record. At each visit the dietician interviewed the mother and used a seven-day history technique to expand the details recorded in the dietary record. The infants' diets were analysed using tables of standard food composition (Thomas and Corden 1977) and information supplied by manufacturers of commercial baby foods. Individual analyses of the cholesterol content of a representative range of commercial and home-prepared foods were done by Dr. P. Nestel (Baker Research Institute, Melbourne).

Calculations of daily energy intake were expressed in Kcal (mJ) per day, Kcal (mJ) per kg body weight per day (Kcal(mJ)/kg/d), and Kcal (mJ) as percentage of the energy intake appropriate for the weight corresponding to the child's height percentile. These

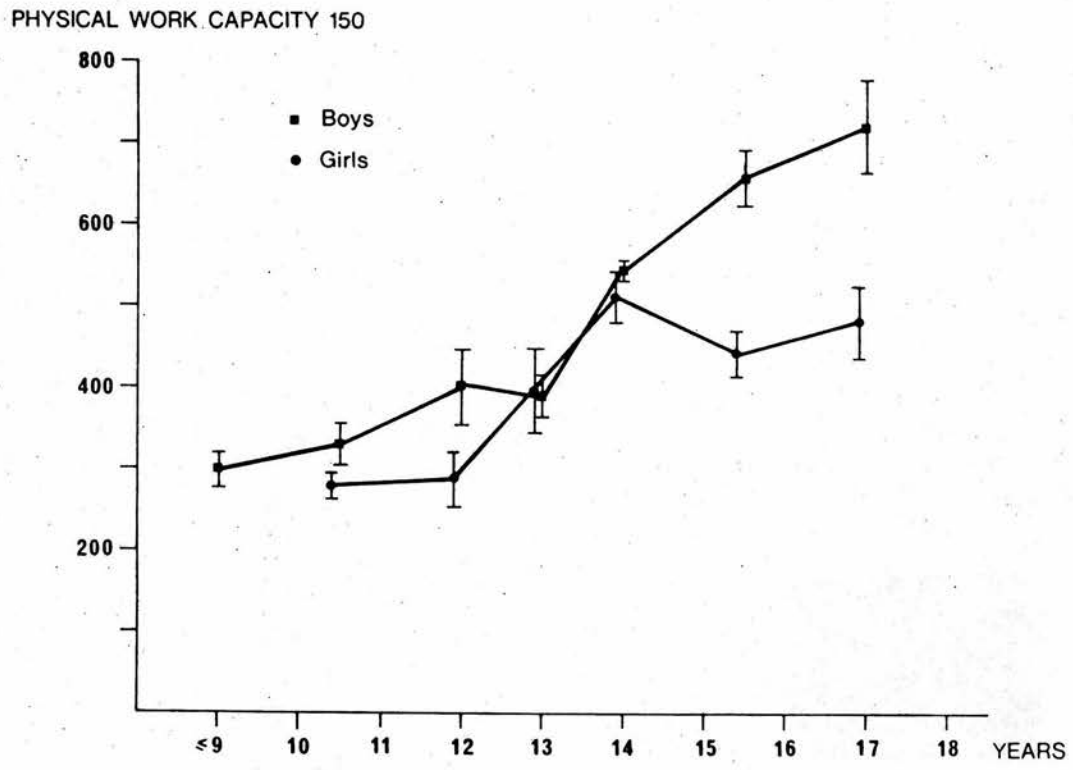

Fig 16. Physical work capacity at a heart rate of 150 beats per min. expressed in Kiloponds per metre per minute for children in the schools sample.

values were obtained using 100 Kcal/kg/d for children of 3 to 10 kg body mass, with an additional 50 Kcal/kg/d for body mass above kg, based on a metabolic rate studies (Winters 1973).

The differential nutrient intake was described as a percentage of the total energy derived from carbohydrate, protein, fat, from milk and transitional solid foods (beikost).

Dietary analyses could not be done for partly breast-fed babies, it being impossible to assess the contribution made by milk to their total energy intake. An average composition (Thomas and Corden 1977) was ascribed to the differential nutrient-intake of those being entirely breast-fed. No reliable estimate could be made for their total energy intake, though this was the subject for a further separate study.

The results for the total energy intake per day for three, six and twelve months are summarised in Figure 17, and Table 3:4 and those for energy in Kcal(mJ)/kg/d are shown in Figure 18. At three months 36.5% received less than 101 Kcal/kg/d, 18.5% between 100 and 110, and 44.5% more than 110 Kcal/kg/d. At both six and twelve months, the corresponding values were 44, 19.5 and 36.5% (Table 3:5). Figure 19 illustrates the distribution of energy intake expressed as a percentage of that calculated to be appropriate for the weight corresponding to the child's height percentile (% appropr. wt. for ht.). At three months 21.5% were receiving less than 90% of this theoretical figure, and 51.6% more than 110%. By six months the values were 30 and 36%, and at one year 24.6 and 41.6% (Table 3:6).

The values for mean, S.D., mode and range, for percentage of energy derived from carbohydrate, protein and fat, for three, six

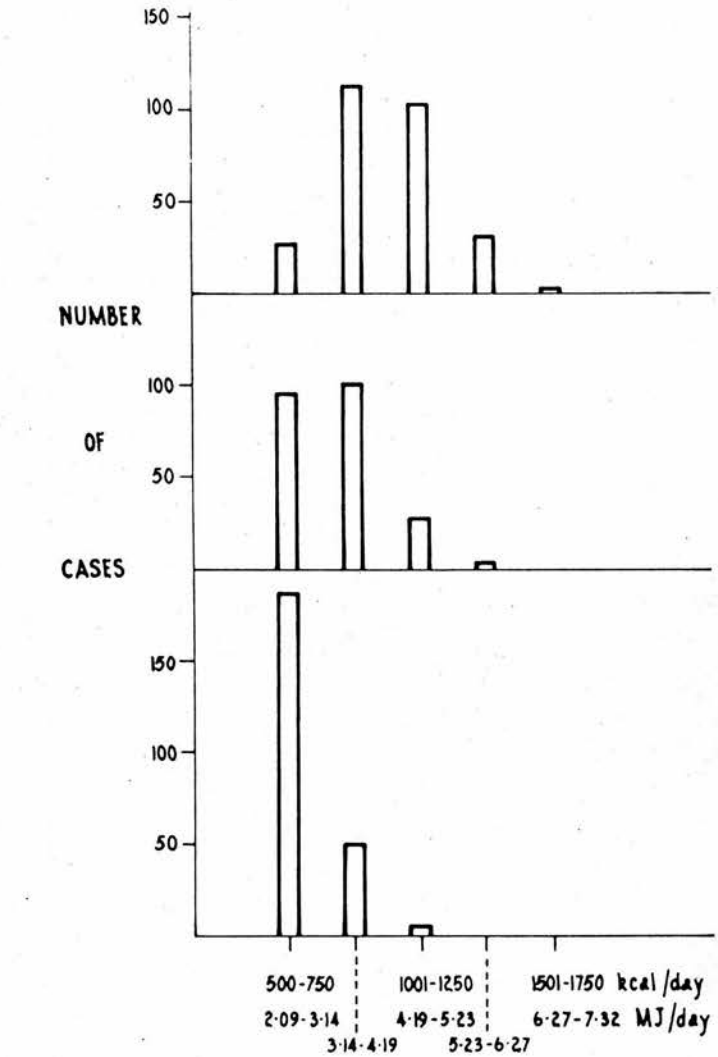

Fig 17. Total daily food energy intake expressed in Kcal and as mJ
Upper panel 12 months
Mid panel 6 months
Lower panel 3 months

TABLE 3:4 Energy intake in Kcal (mJoules) per day for artificially fed infants at 3, 6, and 12 months. Percentage frequency.

Energy intake per day		3 months (271)	6 months (229)	12 months (280)
Kcal	mJoules			
500-750	2.09-3.14	78	42	9.5
751-1000	3.14-4.19	20	44	40.5
1001-1250	4.19-5.23	2	12	36.5
1251-1500	5.23-6.27		2	11.5
1501-1750	6.27-7.32			2

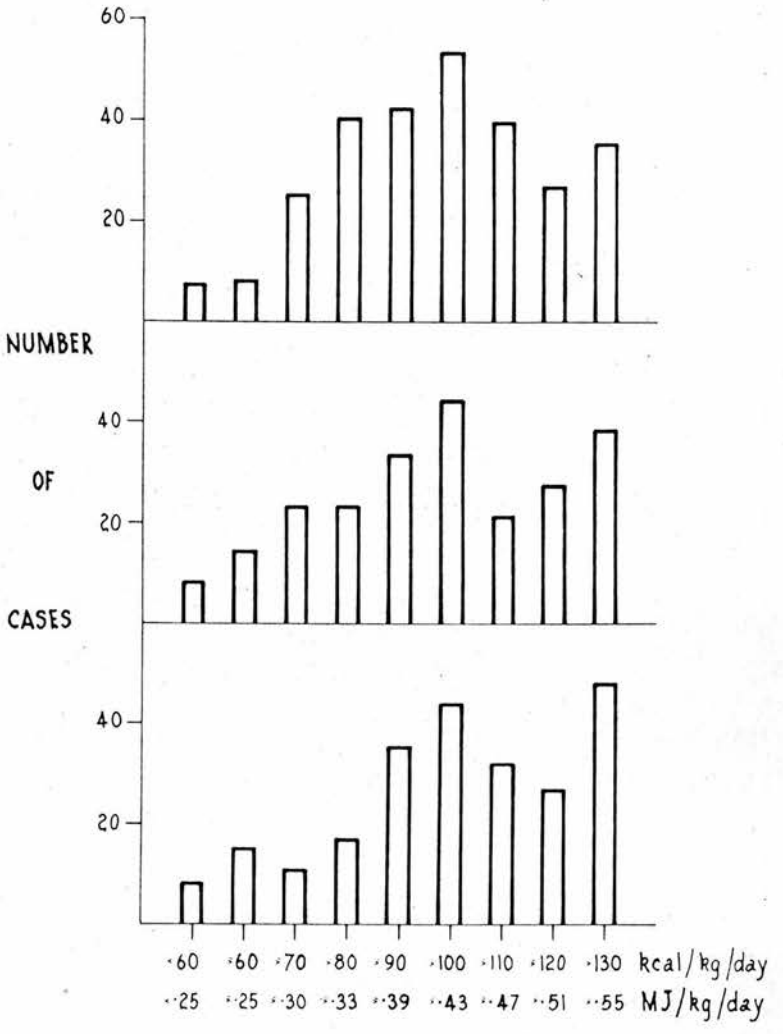

Fig 18. Total daily food intake expressed in Kcal and as mJ per Kg body mass per day

Upper panel 12 months

Mid panel 6 months

Lower panel 3 months

TABLE 3:5 Energy intake in Kcal/kg/d and mJoules/kg body wt/day for artificially fed infants at 3, 6, and 12 months, as percentage frequency, (number).

Kcal/kg/d	mJ/kg/d	3 months (239)	6 months (230)	12 months (277)
<60	<0.25	3.8	3.5	2.5
60-70	0.25-0.29	6.3	6.5	3.3
71-80	0.30-0.33	4.6	10	9.0
81-90	0.34-0.38	7.1	10	14.3
91-100	0.39-0.42	15.0	14.3	15.2
101-110	0.43-0.46	18.4	19.5	19.5
111-120	0.47-0.50	13.4	9.1	14.2
121-130	0.51-0.54	11.3	11	9.4
>131	>0.55	20.1	16.1	12.6

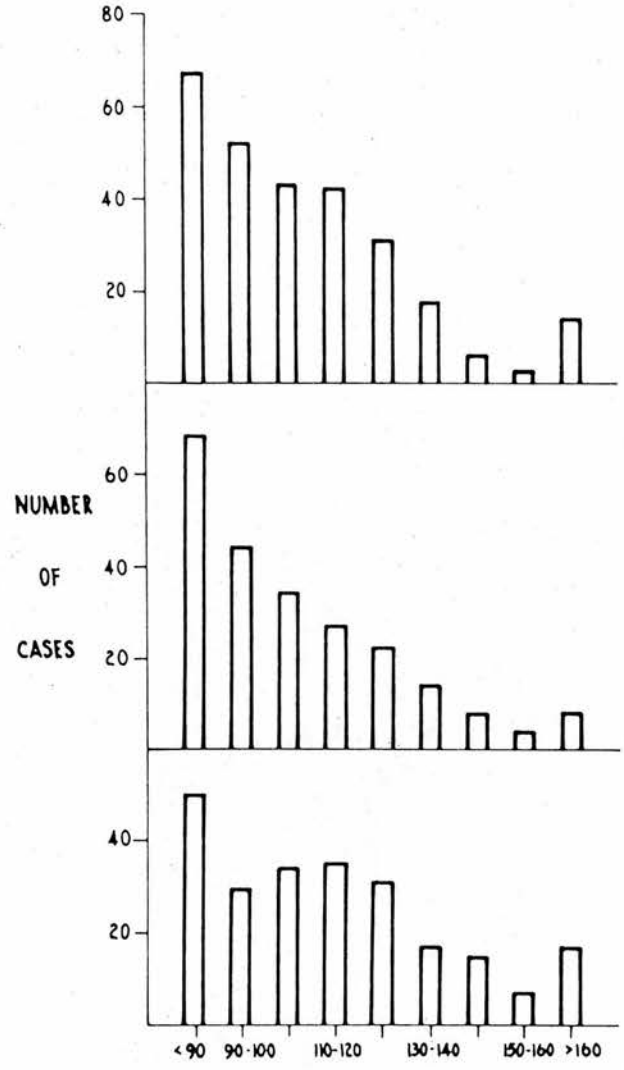

Fig 19. Total daily energy intake expressed as a percentage of that appropriate for body mass for height

Upper panel 12 months

Mid panel 6 months

Low panel 3 months

TABLE 3:6 Energy intake expressed as a percentage of that appropriate for weight for height, as a percentage frequency, (number).

Intake % appropriate at:	Months 3 (237)	6 (230)	12 (270)
<90	21.5	30.0	24.6
91-100	12.7	19.1	18.8
101-110	14.3	14.8	15.0
111-120	14.8	11.7	15.2
121-130	13.1	9.6	11.2
131-140	7.2	6.1	6.2
141-150	6.3	3.5	3.2
151-160	3.0	1.7	0.7
>161	7.2	3.5	5.1

and twelve months, are summarised in Table 3:7. At three months, 81% had a daily cholesterol intake of less than 100 mg; this decreased to 24% at one year. The percentage taking between 100 and 250 mg per day rose from 19% to 57% in the same period (Table 3:8).

The two-year-olds were seen within two weeks of their second birthday, and for the preceding week the mother had been asked to complete a week's record of their child's food intake. Written instructions on the method of weighing their child's food on a kitchen scale were enclosed with the appointment. The mothers recorded which food items were weighed, which were measured using standard kitchen measuring utensils, and which were estimated. These dietary records were discussed with each mother by the dietician at the visit, and special care was taken to determine the intake of extra snack foods. As all the mothers had taken part in the nutritional studies during their child's infancy, they were therefore, to some extent, "trained" in the method used. Care had been taken during previous interviews not to influence the mothers' dietary habits, although by their child's second birthday most of the parents were clearly motivated to co-operate, and this may have had an ill-defined effect on the results of the food records.

From the dietary records analyses were made of the protein, fat, carbohydrate, cholesterol, and food energy contents using standard tables (Thomas and Corden 1977) and additional information received from manufacturers about individual food items. Calculations of energy intake were made using the Atwater factors of 4 Kcal (16.74 KJoules) per gram of protein and carbohydrate,

TABLE 3:7 The percentage of energy derived from carbohydrate, protein and fat at three, six and 12 months.

	Mean	S.D.	Mode	Range	n
CARBOHYDRATE					
3	45.3	6.8	42	30-70	322
6	49.1	8.6	42	30-76	239
12	49.3	8.2	44	31-79	279
FAT					
3	42.5	7.2	50	20-61	322
6	36.4	7.0	30	11-60	239
12	34.9	6.1	34	14-50	279
PROTEIN					
3	12.2	4.4	7	4-21	322
6	14.5	3.7	15	5-22	239
12	15.8	2.8	16	4-25	279

TABLE 3:8 Cholesterol intake (mg/day) at 3, 6, and 12 months as percentage frequency, (number).

	3 months (391)	6 months (320)	12 months (285)
100	81	62	19
100-250	19	35	57
251-500		3	21
501-750			2
>750			1

and 9 Kcal (37.67 KJoules) per gram of fat.

The values for the daily intakes of food energy, (as mJ and as mJ per kg body weight), cholesterol, and the percentage contribution to the diet from protein, carbohydrate, and fat, are shown on Table 3:9.

The boys had a higher modal daily energy intake than the girls: 1500 Kcal (6.29 mJ) versus 1000 Kcal (4.18 mJ), although their mean values were similar (1215 Kcal v 1129 Kcal/day) (0.51 mJ v 0.54 mJ). There were no significant differences between the sexes for the contribution to total daily energy intake from protein, carbohydrate, or fat.

Thirty-three children had results that were above the 97th percentile or below the 3rd percentile for energy intake, cholesterol intake, and/or the percentage contribution to energy from protein, fat, or carbohydrate (Table 3:10). Of these thirty three children, 48% had complete seven-day dietary records and 6% had records of 1-4 days; of these, 63% had weighed the food. The remaining 46% of the diets were calculated from histories taken by the dietician. Three children had an alcohol intake which provided from 3% to 8% of their daily energy intake.

School sample.

A four day dietary record was kept by each child in which was recorded every food or drink item consumed. The amounts of food and volumes of fluid were described in kitchen unit, or pre-weighed. This record was supplemented with information taken during an interview with the child concerning the extra food items which had been eaten but not recorded. These included soft drink, ice-cream, sweets, chips etc. which had usually been purchased with lunch or pocket money.

TABLE 3:9 Values (mean ± SD) at two years for daily intake of energy (megajoules), cholesterol (mg/day), and the percentage of daily food energy derived from protein (% protein), carbohydrate (% CHO) and fat (% fat).

	Total (n = 197) sample		Boys (n = 102)		Girls (n = 95)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Energy intake (mJ/day)	5.02 ± 1.86	1.93-7.70	5.08 ± 0.99	2.64-7.70	4.73 ± 1.03	1.93-7.12
mJ per kg body weight	0.40 ± 0.14	0.13-0.64	0.40 ± 0.88	0.23-0.56	0.39 ± 0.09	0.13-0.64
Cholesterol (mg/day)	199 ±93.56	50 - 610	199 ±86.42	50 - 480	196 ±100.48	50 - 610
% CHO	47.3 ± 7.34	22 - 73	47.2 ± 7.64	22 - 73	47.6 ± 7.08	32 - 73
% Protein	14.1 ± 2.28	7 - 20	14.0 ± 2.30	7 - 19	14.1 ± 2.30	7 - 20
% Fat	38.7 ± 5.82	20 - 59	38.9 ± 6.07	20 - 59	38.3 ± 5.58	20 - 53

TABLE 3:10 The 3rd and 97th percentiles for nutrient data for boys and girls at two years.

	97th		3rd	
	Boys	Girls	Boys	Girls
Energy intake in Kcal (mJ)	1620(6.78)	1560(6.53)	785(3.29)	700(2.93)
Dietary cholesterol (mg/day)	390 mg	370 mg	-	-
Percentage energy from carbohydrate	61%	62%	32%	34%
Percentage energy from protein	17%	18%	9%	10%
Percentage energy from fat	49%	47%	20%	25%

Values for nutrient intake were calculated from the tables of composition of Australian Foods (Thomas and Corden 1977), and from information obtained from manufacturers. Standard Atwater factors were used to calculate energy intakes: 4 Kcal (16.74 kJ) per gram for protein and carbohydrate, 7 Kcal (29.3 kJ) per gram for alcohol, and 9 Kcal (31.67 kJ) per gram for fat.

The results for daily food energy intake for each age group studied are shown in Figure 20, and the results have been expressed against both chronological (CA) and bone age (BA) in Figure 21. The results for the percentage contribution to the total daily food energy intake from protein, carbohydrate, and fat, and the daily cholesterol intake, are shown in Figure 22. There was little difference between boys and girls for these latter measurements, although the boys' cholesterol intake increased steadily with age and remained higher than the girls' intake.

The total daily energy intake was consistently higher for boys at each age, rising from 8.93 to 11.84 mJ as compared to the girls' decrease in daily intake from 7.68 to 6.14 mJ by 17 years CA. The results were plotted for BA to see if this more accurate measurement of somatic maturity would clarify the changes in food intake occurring at this time of rapid somatic development. From Figure 25 it can be seen that the change in food intake expressed against CA follow by six months the changes occurring against BA while for the boys it can be seen that whilst there was a step-wise increase in food intake occurring from 12 years CA this increase was rapid and uninterrupted from 13 years to 16 years BA.

The validity of the results of these nutritional analyses depends both on the accuracy of dietary histories, and on the tables

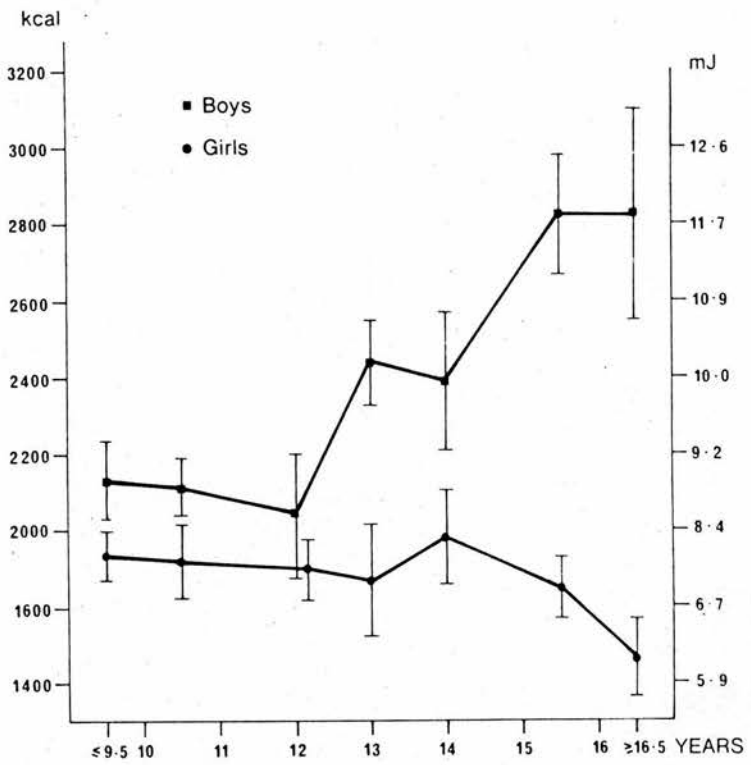

Fig 20. Daily total energy intake (Kcal and as mJ) for each age group in the schools sample.

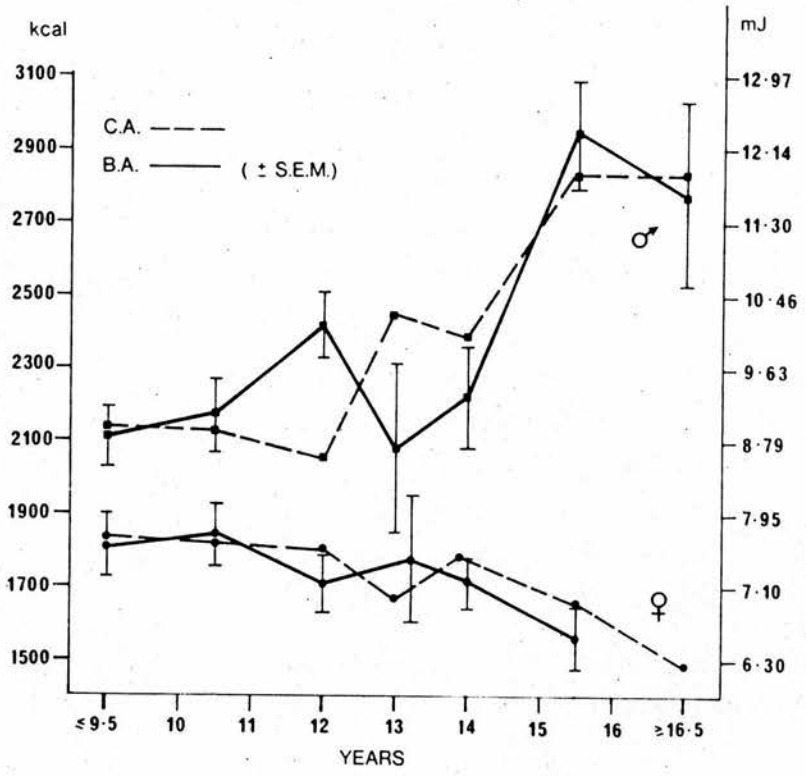

Fig 21. Daily total energy intake (Kcal and as mJ) for both chronological (CA) and bone (BA) age groups in the schools sample.

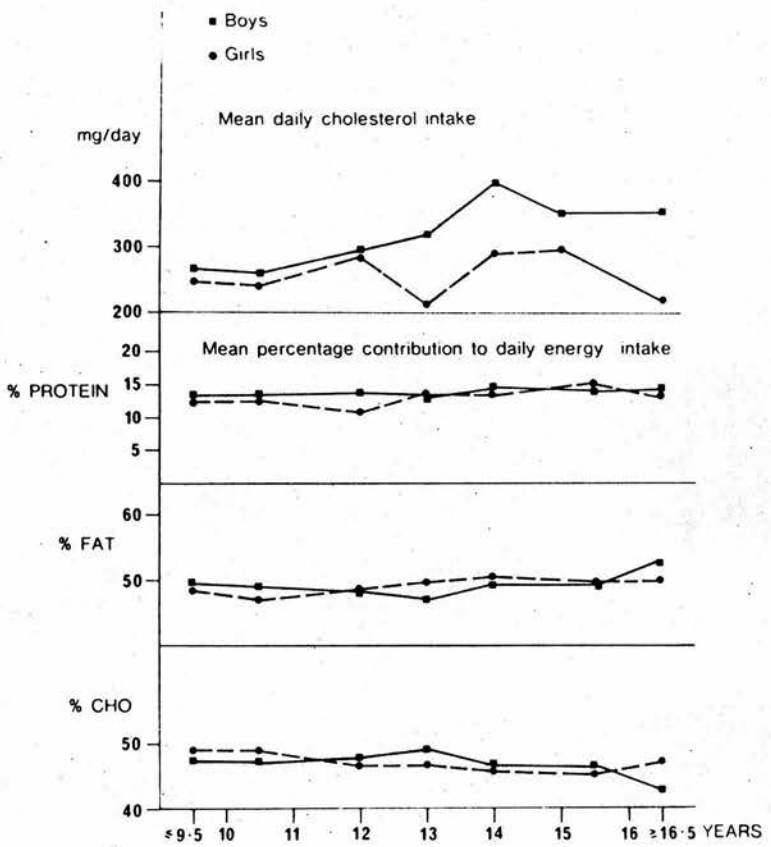

Fig 22. The percentage contribution to daily food energy intake from protein, fat, and carbohydrate, and the daily cholesterol intake (mg) in the schools sample.

of food composition on which the calculations were based. In such epidemiological studies there is a dilemma between increasing the accuracy of data collection at the expense of the practicability and feasibility of the study which itself will be reflected in the co-operation of the subjects. However, there is acceptably good agreement between the 24 hour recall, the diet diary, and the food record in which each food item is weighed (Macleod 1972; Blake and Durnin 1963, McClean and Weston 1976) and awareness of potential errors may minimise them (Fidanza 1974). These include both qualitative and quantitative recording errors, memory distortions causing omissions from the diary of food thought to be unacceptable to the dietician, and under or over-estimating food items which happen to be under or over represented on the days on which the diary is kept. For the infants in this study a balance between accuracy and mothers' compliance was sought by using a 48-hour food diary supplemented by a 24-hour food intake recall conducted by the dietician at each visit. This method has been shown to correlate well with the 7-day food record (Young et al 1952a, McClean and Weston 1976), and using a large sample shorter food histories give as accurate a picture of the population's nutritional intake as do longer histories on smaller samples (Young et al, 1952b). Using a large sample, systematic errors are less likely to exert their influences, being obscured by the presence of a greater proportion of random errors. It was also considered that the natural monotony of the diet during early infancy with little day-to-day variation, and the mothers becoming used to the method of recording food intake through the year as the children's diets became more varied, would also have reduced the

potential for error.

For the two-year-olds a week's dietary record was used in which food was weighed and liquids measured. This was found to be a practical and accurate method (Young et al 1952 a, b., Macleod 1972, Allen et al 1977), and was combined with a food history taken by the dietician which allowed the nutrient intake over a weekly shopping period to be calculated. Four days was found to be the limit of acceptance by many mothers, and this duration has been shown to give as satisfactorily accurate results as a longer period (Gersovitz et al, 1978). This method was used for the school children, encompassing two school days and a weekend.

Other potential sources of error may be found in the individual variations in the assimilation of nutrients taken, and the possible lack of correspondence between food tables and the actual composition of the foodstuffs (Fidanza 1974). Although babies show individual variations in the proportion of nutrients, and in particular fat absorbed and hence the losses of energy through the faeces (Southgate and Barrett 1966), these are impossible to quantitate outside a metabolic ward (Ounsted and Sleigh 1975, Widdowson 1965). They were therefore disregarded, and standard Atwater factors used (Southgate and Durnin 1970).

CHAPTER 4

CHOLESTEROL FROM BIRTH TO MATURITY

INTRODUCTION

The data in this chapter concerns the values for serum total cholesterol (TC), low (LDL-C) and high (HDL-C) density lipoprotein cholesterol, and triglyceride at each age group for the children described in these studies, and the methodology used in the analyses including the rocket immunoeletrophoretic method used for measuring apolipoprotein B in a sample of two-year-olds' serum.

The samples: these have been described in Chapter 2.

Methodology for cholesterol analyses

For the cord blood samples the umbilical cord was routinely clamped within 1 min of delivery and 5 to 10 ml of mixed blood was taken from the placental end of the cord prior to delivery of the placenta. The serum was separated and stored at $4^{o}C$ within 12 hours of delivery.

The blood samples from the children aged three months to two years were taken by thumbprick by an expert technologist. These children were not fasting. The four-year-olds were fasting, and also had thumbprick samples taken.

Approximately 0.5 to 1 ml blood only was required for the complete analyses.

The school children had venepuncture samples taken after an overnight fast, with minimal vein occlusion.

The serum cholesterol was measured using the enzymatic method of Allain et al (1974), and the reagent manufactured by Abbott Laboratories

(Abbott Laboratories, Diagnostic Division, 820 Mission St., So Pasadena, CA 91030). Analyses were performed on a CentriChem (Union Carbide, distributed by Hoffman-La Roche, Basel), using 10 µl serum. A reference serum supplied by the Centre for Disease Control Laboratory, Georgia, was used as a primary standard.

HDL-C was prepared by precipitation of other lipoproteins with heparin-manganesechloride (Lutmer et al, 1974). The final concentration of heparin solution was 183 anticoagulability units/ml, which resulted in complete precipitation of non-HDL lipoproteins (Backorick et al, 1976). This was confirmed by the disappearance of non-HDL lipoprotein bands on polyacrylamide-gel electrophoresis following heparin-manganese precipitation. The HDL-C fraction and the second aliquot of serum of the cord sample was frozen at -20°C prior to lipoprotein assay, with the HDL-C fraction being centrifuged to remove the particulate matter which appeared on thawing. The specimens from the other samples were all analysed when fresh. A small separate comparative study has demonstrated no difference between the values for HDL-C obtained on fresh serum or after freezing, thawing, and centrifugation of the previously precipitated sample (Table4:1).

Artefactually high values for HDL-C may occur when a manual enzymatic method is employed to estimate cholesterol in HDL fractions precipitated with manganous salts, for a fine precipitate develops during the reaction (Steele et al, 1976). Reconstitution of the enzyme reagent in ethylene diamine tetra acetic acid prevents this. Trials using the CentrifiChem centrifugal analyser showed no difference between cholesterol values whether or not ethylene diamine tetra acetic acid was used, because the fine precipitate

TABLE 4:1 The effects of freezing and thawing on HDL cholesterol values (n=23).

	Mean mmol/L	SD	p*
HDL extract prepared from fresh serum, analysis same day	0.69	0.27	>0.1
HDL extract prepared from frozen and thawed serum	0.66	0.23	>0.1
HDL extract prepared from fresh serum, then frozen, thawed, and analysed	0.33	0.21	<0.001
HDL extract prepared from fresh serum, then frozen, thawed, centrifuged and analysed	0.70	0.25	>0.1

* value based on comparison with fresh serum

which formed in the absence of ethylene diamine tetra acetic acid was centrifuged out of solution during the reaction.

The value of LDL-C was derived using the formula LDL-C = TC-(TG/5 + HDL-C). The results obtained in this way have been shown to correlate well with those obtained by ultra-centrifugation (Glueck et al, 1973).

Triglycerides (TG) were measured by an enzymatic method on Centrifi-Chem on 10 µl serum, using Calbiochem Triglyceride-Glycerol-Stat-Pack (Calbiochem, California). A reference serum with an assigned value was used as a standard. The assigned value was determined using a manual enzymatic method (Calbiochem) with pure triolein as primary standard (Chong-Kit et al, 1974).

Duplicate quality control samples were included in each run to assess precision. One was placed in a fixed position, the other in a random position on the sample tray. There was no significant difference between the results for quality control samples in fixed and random positions. Mean values and standard deviations for the quality control samples were:-

Cholesterol (mmol/l);	1.82 ± 0.09	(C.V. 5.04%)
	3.19 ± 0.09	(C.V. 2.9%)
	4.90 ± 0.12	(C.V. 2.4%)
	5.30 ± 0.11	(C.V. 2.08%)
Triglyceride (mmol/l);	0.25 ± 0.01	(C.V. 4.8%)
	0.59 ± 0.02	(C.V. 3.6%)
	0.95 ± 0.03	(C.V. 3.2%)
	1.58 ± 0.05	(C.V. 3.16%)

Methodology for apolipoprotein B (ApoB) analysis.

Estimations of serum apoB concentrations were performed on 82 mothers, 38 fathers, and 84 two-year-olds. Of these there were 38 complete families (analyses for both parents available). This sample population was randomly selected on the basis of the adequacy and availability of the specimen volumes. Serum was obtained from fasted adults overnight, whereas the children had not been fasted.

ApoB was measured by rocket immunoelectrophoresis (RIE) using a modification of the method of Laurell (1966). Serum sample were diluted 1/20, and 10 µl aliquots loaded into 4 mm diameter wells. The electrophoresis was carried out in barbital buffer pH 8.6 for 18 hours overnight, with a potential difference of 2V per cm in the gel. The gel contained 2% agarose, 5% Dextran T10, sodium azide 0.001% and monospecific antibody to human apoB. The monospecific antiserum used was prepared in rabbits by injecting human LDL of density 1.019-1.055 g/ml which had been washed twice after initial isolation by ultracentrifugation at 39,000 rpm for 22 hurs in a Spinco model L3-50 ultracentrifuge at $8^{o}C$. The identification of the protein is given by the rocket shaped precipitate formed. This was visualised by staining with Coomassie Brilliant Blue R-250 after the gel had been washed and dried. LDL of known protein concentration was used for a standard curve and aliquots of a human serum of known apoB concentration were run on each plate for further standardisation. A commercial human standard β-lipoprotein serum was also used (Behring Institute, containing 484 mg/100 ml). Protein was estimated using the method of Lowry et al (1951). Calculations were based on the height of the rocket and gave the same results as estimating the area

of the rocket. Intraplate variation was <5% and interplate variation <10%.

In order to test the efficacy of the rocket technique, 22 fresh serum samples, selected at random from children not in the study, were collected and analysed on the same day for TC, LDL-C, HDL-C. and TG and for apoB using the rocket technique. The correlation between TC and apoB, and LDL-C and apoB was 0.87 in both cases.

RESULTS

The values for TC, LDL-C, HDL-C, and TG, are presented in Tables 4:2 to 4:5 with the percentiles according to age shown in Figures 1 to 8.

The TC levels peaked at one year for girls and nine months for boys, then declined to two years. From four years the levels rose by approximately 12%, and (allowing for the gap until 9 years) remained steady until 13 years. The levels then increased to, and remained at, adult levels; there being no persistent rise through teenage to adulthood except for those in the upper percentiles. This pattern of change was similar for both LDL-C and HDL-C. Because the infants' and two-year-olds were not fasting, no comment can be made on their TG levels in relation to the school children's, although the TG levels showed a steady increase through puberty to late teenage.

The girls' TC level was slightly higher than the boys' to two years, but from four years there was no consistent sex difference with the overall mean values for the school children being nearly identical at 4.95 ± 0.96 for girls and 4.92 ± 0.95 mmol/l for the boys. No consistent sex difference was present for either HDL-C, LDL-C or TG levels.

TABLE 4:2 Serum total cholesterol values (mmol/L) for each group studied (m = male, f = female).

	Sex	Mean (mmol/L)	S.D. (mmol/L)	Range (mmol/L)	N
Cord	m				
	f	1.83	0.56	0.59 - 3.74	1926
3 months	m	4.16	0.92	0.54 - 8.5	180
	f	4.32	0.84	2.26 - 6.81	183
6 months	m	4.28	0.84	1.17 - 7.74	160
	f	4.39	0.95	0.57 - 6.99	158
1 year	m	4.28	0.84	1.97 - 6.6	140
	f	4.52	0.91	2.26 - 7.87	137
2 years	m	4.08	0.78	2.39 - 6.11	99
	f	4.20	0.68	2.39 - 6.08	95
4 years	m	4.70	0.82	2.99 - 5.95	197
	f	4.69	0.83	1.30 - 7.74	188
9 years	m	4.71	0.86	3.27 - 6.57	16
	f	4.53	0.82	2.93 - 5.92	17
10.5 years	m	4.72	1.22	2.34 - 7.59	23
	f	4.74	1.05	3.12 - 7.15	22
12 years	m	4.79	0.69	4.03 - 6.00	8
	f	4.58	0.76	2.60 - 5.72	15
13 years	m	4.94	0.89	3.79 - 6.73	16
	f	5.35	1.10	3.38 - 7.12	15
14 years	m	4.82	0.91	3.17 - 6.39	12
	f	4.56	0.85	3.56 - 6.18	7
15.5 years	m	5.16	0.81	3.9 - 7.41	24
	f	5.26	0.86	3.9 - 7.35	27
17 years	m	5.29	1.04	3.92 - 7.95	15
	f	5.40	0.81	4.18 - 7.07	11
Combined schoolage	m	4.92	0.95	2.35 - 7.95	116
	f	4.95	0.96	2.60 - 7.35	114
Adults	m	5.18	1.24	1.92 -10.29	121
	f	4.70	0.94	1.69 - 8.60	189

TABLE 4:3 Serum low-density lipoprotein cholesterol values (mmol/L) for each age group studied (m = male, f = female).

	Sex	Mean (mmol/L)	S.D. (mmol/L)	Range (mmol/L)	N
Cord	m, f	0.90	0.49	0.28 - 3.30	1797
1 year	m	2.27	0.82	0.2 - 4.57	131
	f	2.46	0.91	0.31 - 5.74	120
2 years	m	2.02	0.73	0.28 - 3.56	95
	f	2.20	0.30	0.85 - 4.62	84
4 years	m	2.62	0.94	0.33 - 5.98	135
	f	2.65	0.86	0.31 - 5.33	144
9 years	m	2.80	0.74	1.66 - 4.57	16
	f	2.68	0.81	1.32 - 4.05	17
10.5 years	m	2.93	1.14	1.11 - 5.66	21
	f	2.98	1.01	1.17 - 5.53	22
12 years	m	3.01	0.82	2.1 - 4.39	18
	f	2.60	0.79	0.49 - 3.66	15
13 years	m	2.96	0.78	1.53 - 4.42	16
	f	2.96	0.96	1.24 - 4.36	15
14 years	m	2.71	0.96	0.78 - 4.34	12
	f	2.55	0.68	1.61 - 3.74	7
15.6 years	m	2.69	1.01	0.98 - 5.04	24
	f	2.81	0.88	1.66 - 5.33	26
17 years	m	2.93	0.98	1.48 - 5.22	15
	f	3.01	0.81	1.87 - 4.81	11
Combined schoolage	m	2.83	0.93	0.78 - 5.67	114
	f	2.82	0.88	0.49 - 5.54	113
Adults	m	3.05	0.98	0.44 - 5.95	102
	f	2.70	0.93	0.44 - 8.06	174

TABLE 4:4 Serum high-density lipoprotein cholesterol values (mmol/L) for each age group studied (m=male, f=female).

	Sex	Mean (mmol/L)	S.D. (mmol/L)	Range (mmol/L)	N
Cord	m, f	0.70	0.33	0.02 - 2.88	1848
1 year	m	1.07	0.38	0.26 - 2.34	135
	f	1.06	0.41	0.26 - 2.34	124
2 years	m	1.36	0.57	0.57 - 5.85	97
	f	1.30	0.57	0.62 - 5.95	92
4 years	m	1.66	0.65	0.72 - 4.29	134
	f	1.68	0.67	0.80 - 5.95	144
9 years	m	1.59	0.37	0.91 - 2.05	16
	f	1.50	0.26	1.09 - 1.92	17
10.5 years	m	1.65	0.32	10.1 - 2.31	23
	f	1.35	0.32	0.80 - 1.92	17
12 years	m	1.43	0.26	1.04 - 1.87	8
	f	1.69	0.32	1.06 - 2.26	15
13 years	m	1.60	0.36	0.85 - 2.08	16
	f	1.88	0.47	1.19 - 2.83	15
14 years	m	1.68	0.46	1.17 - 2.9	12
	f	1.52	0.32	1.17 - 1.89	7
15.5 years	m	1.94	0.61	1.22 - 3.69	24
	f	1.94	0.38	1.35 - 2.88	26
17 years	m	1.70	0.45	1.01 - 2.93	15
	f	1.82	0.22	1.45 - 2.23	11
Combined schoolage	m	1.70	0.45	0.86 - 3.69	116
	f	1.68	0.40	0.81 - 2.89	113
Adults	m	1.35	0.58	0.46 - 5.59	102
	f	1.49	0.32	0.72 - 2.31	172

TABLE 4:5. Serum triglyceride values (mmol/L) for each age group studied (m = male, f = female).

Age	Sex	Mean (mmol/L)	S.D. (mmol/L)	Range (mmol/L)	N
Cord	m, f)	0.38	0.16	0.10 - 1.46	1916
3 months	m	1.38	0.64	0.02 - 2.83	181
	f	1.39	0.62	0.16 - 3.43	181
6 months	m	1.27	0.59	0.19 - 3.28	160
	f	1.34	0.54	0.20 - 2.83	157
1 year	m	2.00	0.90	0.26 - 4.72	139
	f	2.15	1.03	0.27 - 5.34	137
2 years	m	1.86	0.95	0.46 - 5.04	98
	f	1.70	0.90	0.56 - 5.04	93
4 years	m	0.94	0.36	0.36 - 3.03	192
	f	0.92	0.29	0.34 - 2.70	187
9 years	m	0.73	0.15	0.44 - 0.99	16
	f	0.80	0.23	0.49 - 1.47	17
10.5 years	m	0.75	0.25	0.37 - 1.20	21
	f	0.93	0.55	0.38 - 2.32	22
12 years	m	0.79	0.23	0.57 - 1.27	8
	f	0.67	0.21	0.48 - 1.33	15
13 years	m	0.86	0.30	0.49 - 1.41	16
	f	1.16	0.50	0.51 - 2.11	15
14 years	m	0.98	0.50	0.37 - 2.13	12
	f	1.12	0.49	0.42 - 1.78	7
15.5 years	m	1.49	0.45	0.88 - 2.50	15
	f	1.28	0.22	0.90 - 1.59	11
Combined schoolage	m	0.98	0.42	0.37 - 2.51	114
	f	1.04	0.48	0.38 - 2.79	114
Adults	m	1.48	0.90	0.43 - 5.50	117
	f	1.20	0.88	0.31 - 10.09	187

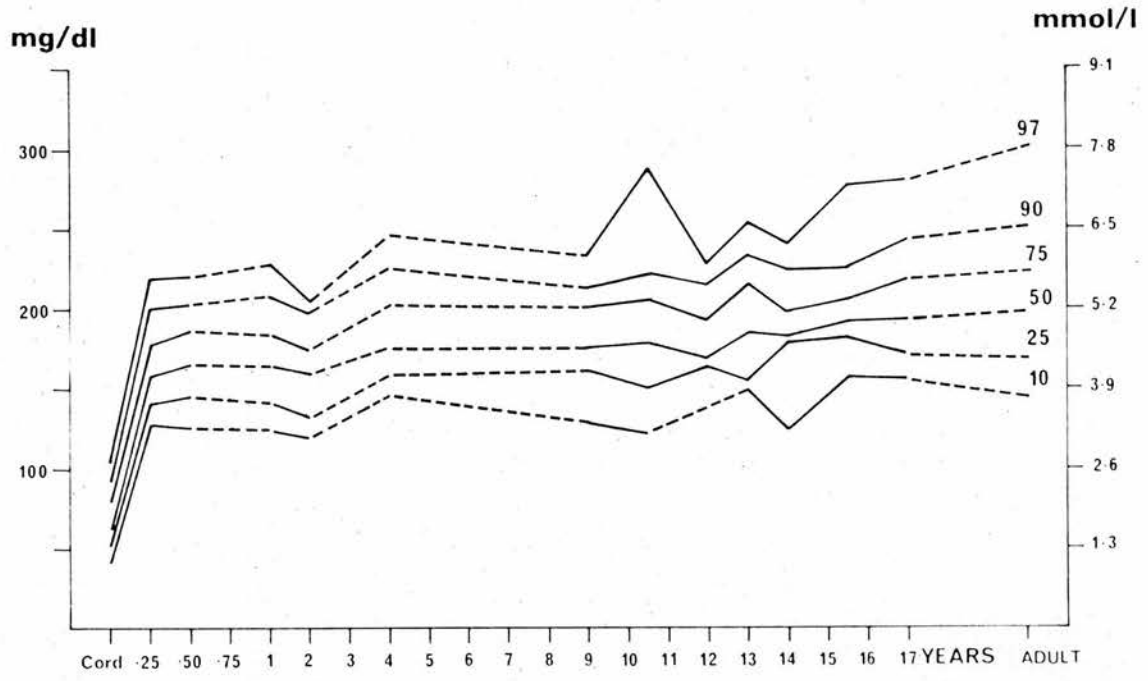

Fig. 1 Percentiles for total cholesterol - males

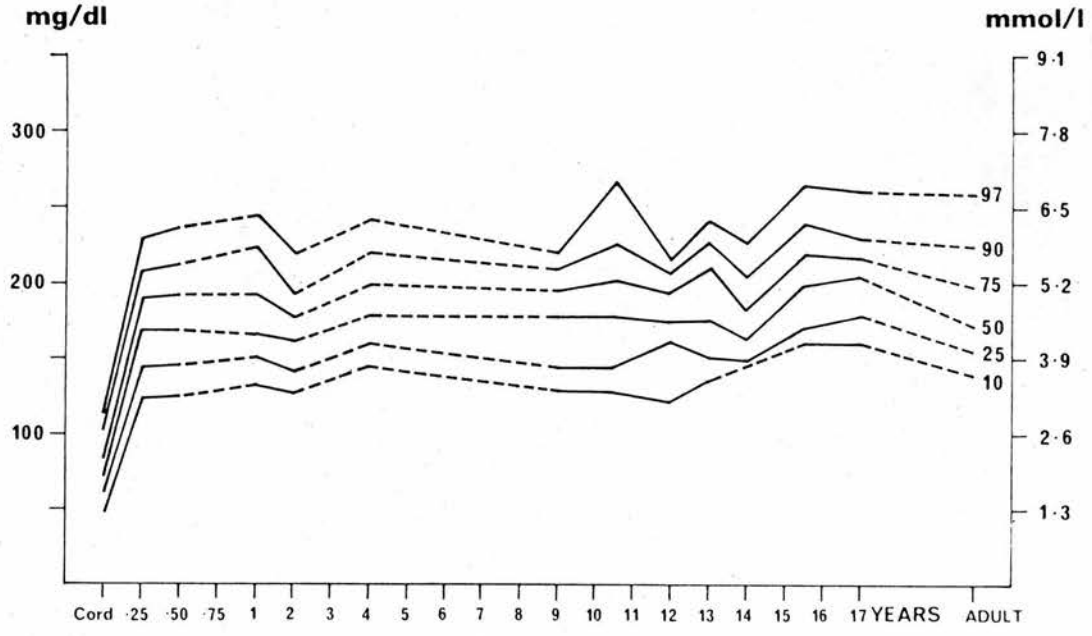

Fig. 2 Percentiles for total cholesterol - females

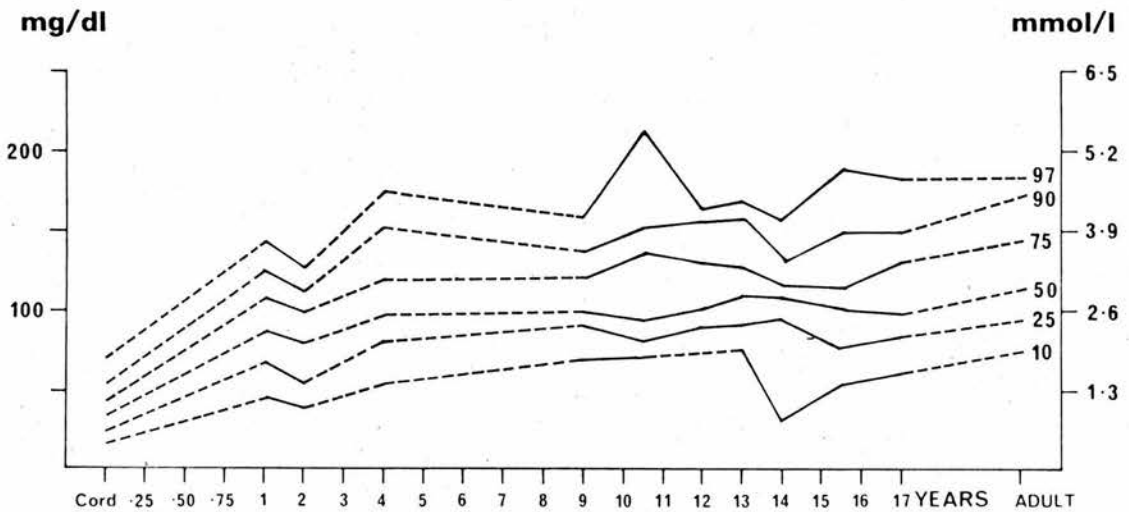

Fig. 3 Percentiles for low density lipoprotein cholesterol - males

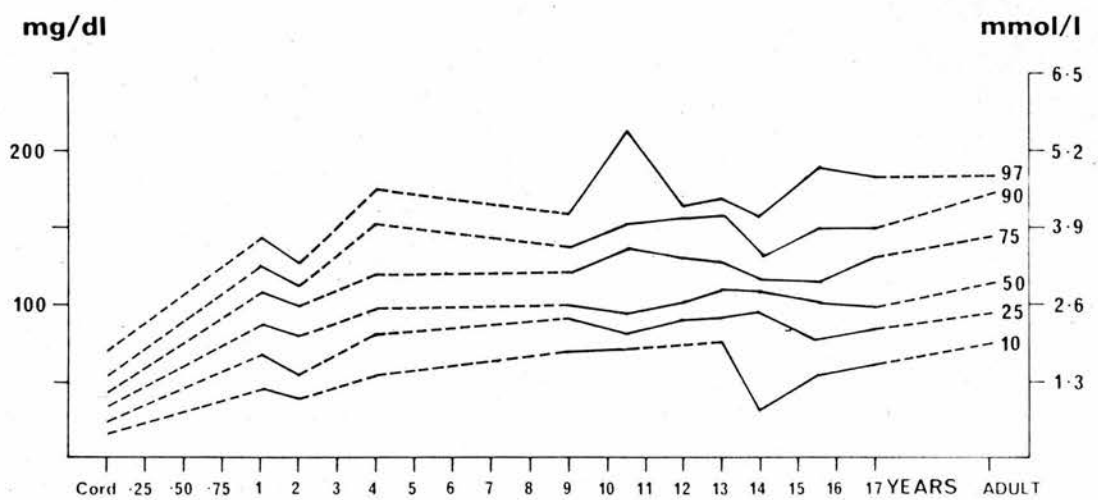

Fig. 4 Percentiles for low density lipoprotein cholesterol - females

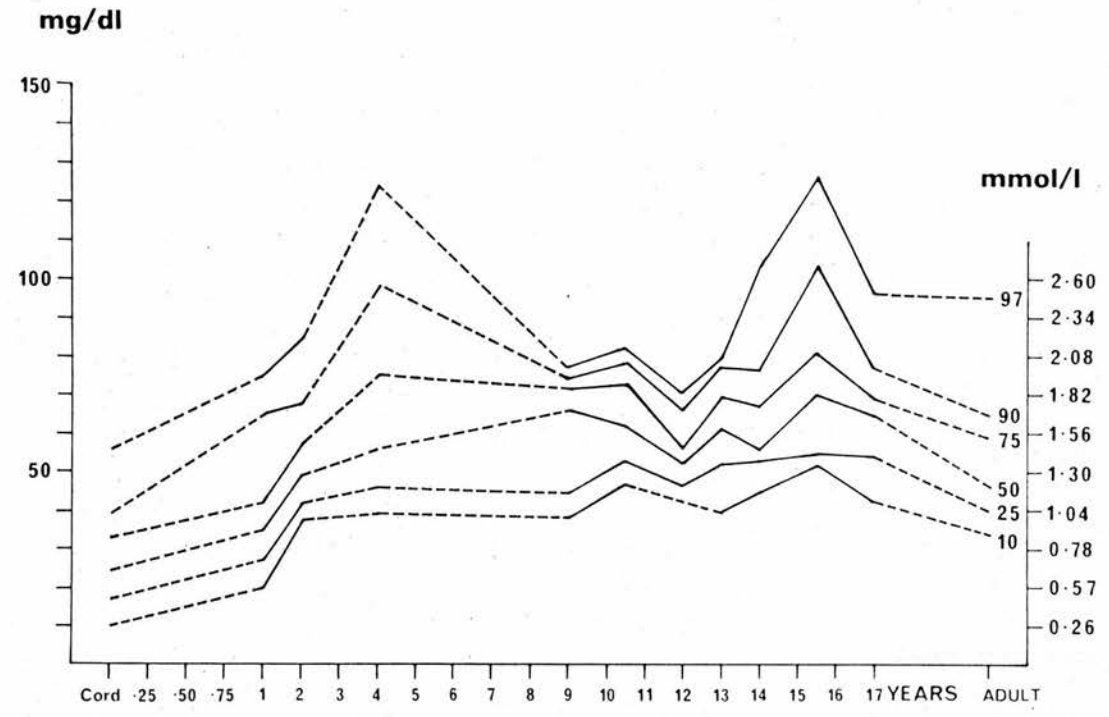

Fig. 5 Percentiles for high density lipoprotein cholesterol - males

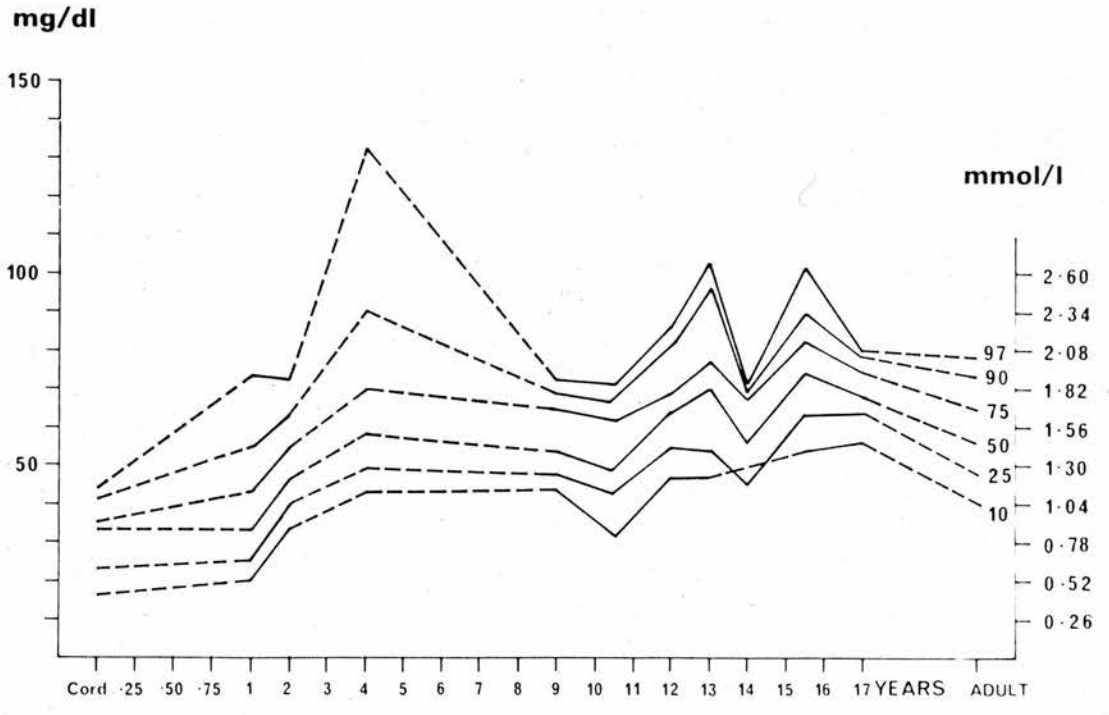

Fig. 6 Percentiles for high density lipoprotein cholesterol - females

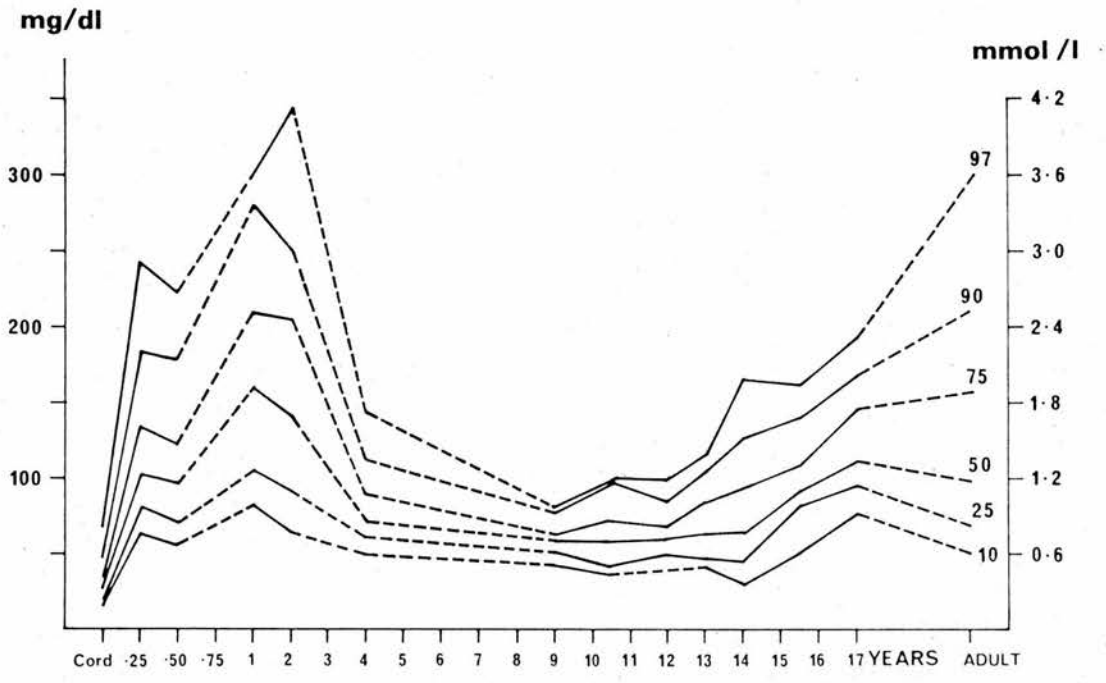

Fig. 7 Percentiles for triglyceride - males

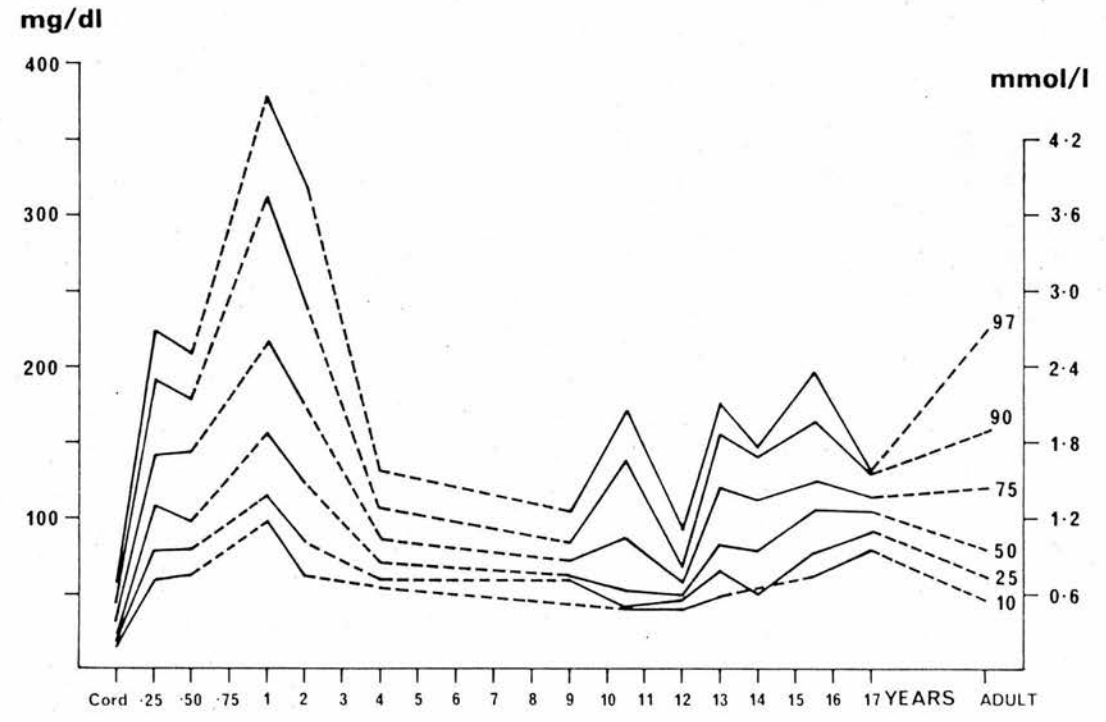

Fig. 8 Percentiles for triglyceride - females

The mean apoB levels and standard deviations for the 87 fathers was 100 ± 35 mg/dl, for the 41 mothers was 98 ± 41 mg/dl, and for the 87 two-year-olds 92 ± 37 mg/dl. The correlations between apoB of the two-year-olds, and the levels of TC, LDL-C, and HDL-C which had been performed on the fresh serum sample were 0.51, 0.53, and 0.43 respectively.

Discussion

This study describes the values for serum total-, low-, and high-density lipoprotein cholesterol, and triglyceride, from healthy children from birth to maturity. Although there were differences in the sample size at different ages, the sample was of adequate size for the longitudinal study to two years, when both nutritional influences are rapidly changing as the child progresses from a milk to a mixed food diet and genetic influences are emerging as determinants of the ranked position for serum cholesterol in the frequency distribution. From age four the lipoprotein levels rose by only approximately one half of a standard deviation to early teenage and subsequently remained at a steady level, with a slight decline occurring for the females into adulthood.

Several immunoassays have been developed for the quantitation of apoB which comprises 98% of LDL protein and 50 to 60% of VLDL protein (Albers et al, 1975; Bautovich, 1975; Bedford et al, 1976). These include rocket immunoelectrophoresis (RIE), radial immunodiffusion in agar or agarose gel and radioimmunoassay. With the development of these techniques, apoB can be estimated directly without using the lengthy and technically difficult procedures of isolating apolipoproteins such as delipidation, gel filtration, and ion exchange chromatography.

In this study using RIE the apoB levels in the parents fell within the range of values reported by Durrington et al (1976) using RIE (88 $\pm$ 11 mg/dl), and of Curry et al (1978), Bautovich et al (1975), and Schonfeld et al (1974) for adult subjects. These authors have not reported apoB values for children. The level of apoB in children (92 mg/dl) was only marginally lower than that found in the adults (98 and 100 mg apoB/dl), suggesting that apoB levels reach equilibrium early on in life. Serum apoB concentrations do not vary as much as TC and TG in the fasting and fed state, therefore fasting the children in epidemiological studies is not necessary (Durrington et al, 1976). The sera analysed in this study had been stored, frozen, for up to six months prior to analysis, and this may have affected the antigenic activity of apoB in serum (Durrington et al, 1976). However, since all serum samples had been treated similarly the absolute value for apoB may have been decreased relative to an estimation using a fresh sample but correlations of apoB between parent and child should not be affected. Further results of the analyses concerning familial inter-relations in lipoprotein using apoB are presented in chapter 7.

Despite nutritional influences being of importance in determining an individual's serum cholesterol level, in a community with a relatively homogeneous diet their effects are masked by a variety of confounding factors including both the naturally occurring seasonal and short-term fluctuations in serum cholesterol (Warnick et al, 1976) and the intrinsic errors of community nutritional analyses (Berwick, 1978). Similarly, although both monogenic (e.g. familial hypercholesterolaemia) and polygenic effects (Carter, 1974;

Heiberg, 1974) on serum cholesterol may be of great significance to an individual by mediating coronary heart disease risk, (Lewis et al, 1974a), their overall effect on a population's cholesterol level is subsidiary to the nutritional effects of which the significance can only be seen in comparative studies.

There is no comparable Australian data on serum lipoprotein cholesterol and triglyceride levels which span the age range reported here. The levels reported from other studies on narrower age groups from Sydney (Hickie et al, 1975 1977), Melbourne (Court and Dunlop, 1975), and Western Australia (Godfrey et al, 1972), have been included in Tables 1:2 and 1:3 for comparison; in addition to results of studies from children in Norway, (Askevøld 1978), Denmark (Dyerberg and Hjörne 1973; Strunge and Trostman 1978), and Northern America (Berenson et al 1974; Lauer et al 1975; Morrison et al 1978). There were no significant differences between the levels reported in these studies although those from Northern Europe and Australia tend to be marginally (less than one S.D.) higher.

The data in these tables and percentile charts for serum cholesterol defines the observed range of normal values, and by inference the abnormal. However for serum cholesterol the observed normal range for a community does not necessarily coincide with the biologically optimal, and therefore cut-off levels are quite arbitrary but may identify those with a frankly increased risk of CHD from HC. Accumulating evidence suggests that those people HC as children will remain HC as adults (Lauer et al 1977), and it may therefore be valid to define an HC child as carrying an excess risk of CHD, which may frequently be associated with other CHD risk factors in Australian children (Chapter 12).

The cut-off levels, with the percentage of children above this, or its percentile value, derived from the various studies have been included in table 1:2. For this study the school children's 97th percentile level was skewed up to 7.28 mmol/L for the boys by the presence of several very HC subjects. It is proposed that a more realistic cut-off level for practical screening purposes be 6.24 mmol/L. Children with a TC level greater than this should have FH excluded by appropriate family studies, in addition to causes of secondary HC, and appropriate dietary intervention instituted.

CHAPTER 5

SCREENING OF CORD BLOOD LOW-DENSITY-LIPOPROTEIN CHOLESTEROL IN THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA

An elevated serum cholesterol may be due to familial hypercholesterolaemia (FH), which is one of the commonest inborn errors of metabolism (Fredrickson and Levy, 1972). The ability to detect this condition in early life may allow long-term prophylactic measures to be taken in one group of people with an increased risk of premature ischaemic heart disease (Kannel, 1970b). Although the excess risk in FH may be partly independent of the actual level of serum cholesterol (Slack, 1969), early intervention to reduce an elevated serum cholesterol level is becoming therapeutically acceptable (Kwiterovitch et al, 1970; Tsang et al, 1975).

The identification of newborn infants with FH by cord blood lipid analysis has been attempted by several groups of investigators but with conflicting results (Glueck et al, 1971; Darmady et al, 1972; Goldstein et al, 1974; Greten et al, 1973; Kwiterovitch et al, 1973; Tsang et al, 1974; Potter and Nestel, 1976b; Anderson and Friis-Hansen, 1976a). The proportions of false-positive results varied considerably in these studies, and the validity of cord serum LDL-C as a marker for future HC remains unproven.

Tsang (1975) suggested that a "Prospective follow-up of much larger numbers of children, selected by cord blood low-density-lipoprotein cholesterol levels, will be required to definitely assess the question of false-negative cord blood cholesterol levels".

Thus, a prospective follow-up study was planned in an attempt to answer three questions: (1) Does an elevated cord serum cholesterol level predict future hypercholesterolaemia in infancy and childhood? (2) Does LDL-C provide a more sensitive marker for future hypercholesterolaemia than TC? (3) What proportion of children with normal cord serum lipid levels subsequently become hypercholesterolaemic?

The sample and methods.

The sample population comprised those 2000 babies described in chapter 2, and the methodology concerning the cholesterol and triglyceride analyses is described in chapter 4 with the initial cord serum values for the whole sample.

Follow-up studies. Four groups were selected for follow-up, based on the cord serum levels of TC and LDL-C: group 1, LCL-C only above the 95th percentile; group 2, both TC and LDL-C above the 95th percentile; group 3, TC only above the 95th percentile; group 4, the control population with cord serum TC and LDL-C levels below the 95th percentile.

The children selected for follow-up because of elevated serum lipid levels (groups 1, 2 and 3) were reviewed between 4 and 12 months of age. The children in the control population (group 4) were reviewed at the ages of three, six, and 12 months, and a non-fasting blood sample taken for lipid analysis. LDL-C was measured at 12 months in control-group children, and on review in children who had cord HC. The lipid levels of parents were measured by the above techniques following a 12-hour fast.

Results

Of the 2000 babies included in the sample population, suitable serum specimens were obtained from 1926. One hundred and twenty-nine specimens were insufficient for either HDL-C and/or TG analysis, so that 1797 results were obtained for LDL-C. The values for the mean, S.D., and 95th percentile (in mmol/L) were, for TC 1.83 $\pm$ 0.56, 2.86; for LDL-C 0.90 $\pm$ 0.59, 1.82; for HDL-C 0.70 $\pm$ 0.33, 1.27; for TG 0.38 $\pm$ 0.16, 1.53. These values are similar to previous studies (Table 5:1). The frequency distribution curves for the cord serum TC and LDL-C are shown in Figs. 1 and 2, and the values (mean $\pm$ SD) in cord serum and at follow-up for the control and HC groups are shown in Table 5:2). Seventy-three HC neonates were followed up, and 373 control-group children were seen once or more during the first year. At follow-up during infancy, there were no statistically significant differences in the mean values for either TC or LDL-C between any of the HC groups, and the control-group.

HC during infancy was defined as a serum TC and/or LDL-C above the age adjusted 97.5 percentile which lay at 5.9 to 6.0 mmol/L for TC from three months to one year, and 4.26 mmol/L for LDL-C at one year. The 97.5 rather than the 95th percentile was selected because it was considered most probable that children with genetically transmitted HC would fall in the higher range. 310 parents of the control group children had their serum cholesterol measured, and from these samples the mean $\pm$ SD and 97.5 percentile (mmol/L) values for TC and LDL-C were defined: for fathers: TC 5.18 $\pm$ 1.24, 7.93; LDL-C 3.05 $\pm$ 0.98, 4.81: for mothers: TC 4.70 $\pm$ 0.94, 6.80; LDL-C 2.70 $\pm$ 0.94, 6.80; LDL-C 2.70 $\pm$ 0.93, 4.81. 6.50 mmol/L lay at the 88th percentile for fathers and 95th percentile for mothers. Of the

Table 5:1 Values of cord blood TC and LDL-C (mmol/l) and the percentage contribution (%) of LDL-C to TC, from previous studies.

		n	TC	LDL-C	(%)
Darmady et al	1972	(302)	2.03 ± 0.59		(47)
Glueck et al	1971	(1800)	1.66 ± 0.49	0.95 ± 0.55*	(47)
Tsang et al	1974	(60)	1.66 ± 0.34	0.75	(44)
Tsang et al	1975	(11)	1.79 ± 0.34	0.78 ± 0.29	(43)
Greten et al	1973	(1323)	1.56 ± 0.52	0.91	(47)
Andersen and Friis Hansen	1976	(303)	2.11		
Andersen and Nielsen	1976	(33)	1.85 ± 0.39	0.70*	(38)
Goldstein et al	1974	(2000)	2.13 ± 0.52		
Potter and Nester	1976	(203)	1.98 ± 0.52		
This study	1979	(1926)	1.83 ± 0.56	0.90 ± 0.49	(49)

* non-HDL cholesterol

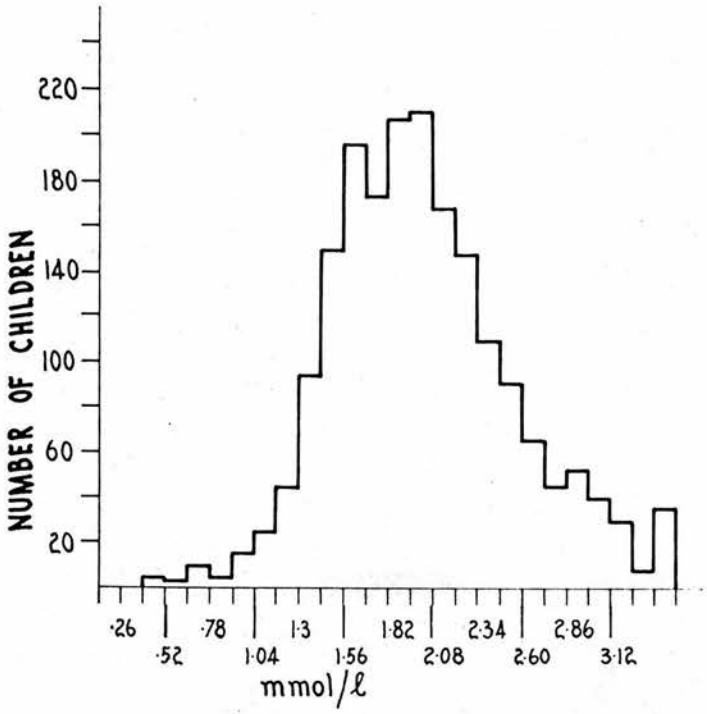

FIGURE 1. Frequency distribution for cord serum total cholesterol

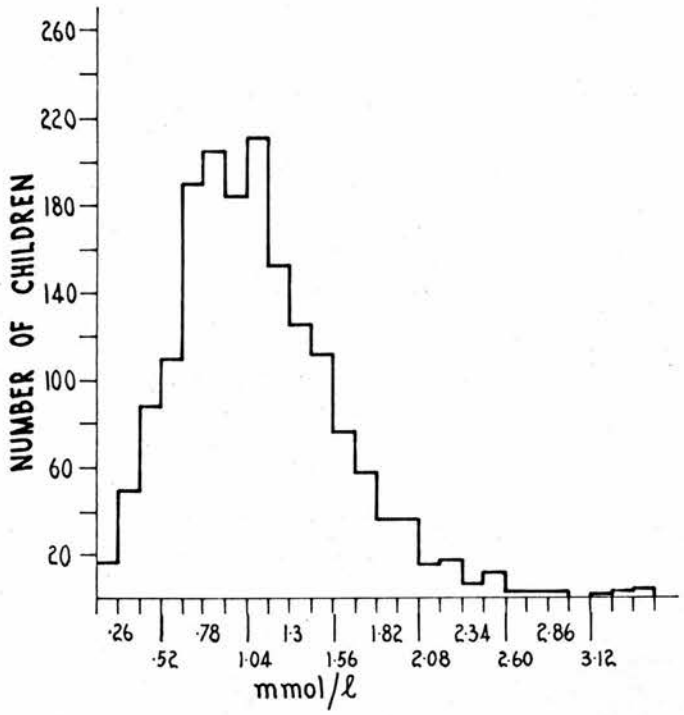

FIGURE 2. Frequency distribution for cord serum low density lipoprotein cholesterol

TABLE 5:2 Values for TC and LDL-C (mean ± mmol/L) in cord serum and at follow-up of the control group children (at one year of age) and of those with cord serum HC (groups 1 to 3 - see text).

Groups	Cord Lipids	Cord serum (x ± SD mmol/L)			Follow-up value (x ± mmol/L)		
		TC	LDL-C	(n)	TC	LDL-C	(n)
1	LDL-C TC	2.95±0.17	2.10±0.16	32	4.31±0.93	2.31±0.98	21
2	LDL-C TC	3.39±0.88	2.31±0.57	49	4.37±1.0	2.39±1.07	28
3	LDL-C TC	3.04±0.19	1.43±0.28	36	4.36±0.75	2.59±1.27	24
4	Control	1.79±0.47	0.87±0.42	330	4.38±0.86	2.33±0.84	272

73 neonates who had had cord serum HC who were reviewed during infancy, HC was detected in six with one child (SM, Table 5:3) having an HC parent. SB's father had moderate HC (6.37 mmol/L, 86th percentile) and his brother also had HC (TC 6.38 mmol/L), their paternal grandfather having died from an infarct at 55 years. No family history of premature ischaemic heart disease was present in other families. SB, SM, and VB had HC persisting from three to six months to one year. The other three children had subsequently normal TC levels, and normo-cholesterolaemic (NC) parents. No information was available about VB's father or his family.

Among the children in the control-group, ten of 354 (2.8%) were HC at three months of age, and all but one child was NC at six and 12 months. At six months, 10 of 310 children (3.2%) were HC and all but one other child were NC at one year. At one year, 8 of 273 (2.9%) were HC, and all were NC on retesting within six weeks. Overall, 27 children were HC during infancy with four having an HC parent (>6.5 mmol/L). None of these adults had a family history of early ischaemic heart disease but two were over-weight males with mixed hypertriglyceridaemia and moderate HC.

False positive and negatives. The follow-up of the HC neonates was incomplete because of lack of interest of the parents, or the families having moved with no forwarding address given. The figures for the false negative and false positive results can therefore only present an approximation of the true picture. One (SM) of the 73 children HC as neonates was HC at follow-up and had an unequivocally HC parent, giving a minimal positive detection rate of 0.05% (1/1926). The false positive rate, referring to those children with cord HC, and subsequent NC was 3.7% (71/1926). False-negative rates refer to

TABLE 5:3 Children with elevated cord serum TC, LDL-C, and hyperchlolesterolaemia at follow-up.

Subject	Cord TC	Cord LDL-C	3 months TC	6 months TC	1 year TC	1 year LDL-C	Father TC	Father TG	Mother TC	Mother TG	Family History early CHD
L.R.	2.96	1.82	4.78	5.20	6.40	5.75	4.81	1.81	4.13	0.95	Nil
I.S.	2.99	2.29	4.50	6.32	4.68	2.86	5.12	0.76	3.82	0.55	Nil
V.B.	3.56	2.13	6.71	-	6.21	3.59	-	-	4.81	0.71	No history
T.F.	3.25	1.95	-	9.31	5.15	3.09	5.82	0.94	5.07	0.52	Nil
S.B.*	3.12	2.11	-	6.01	6.37	4.21	6.37	1.02	5.23	1.02	P.G.G.Fa.
S.M.*	2.68	1.90	-	5.93	6.37	4.21	7.80	3.11	4.94	-	No history

* hypercholesterolaemic parent (TC ≥6.5 mmol/L)

those in whom the serum TC and/or LDL-C was normal in cord blood but elevated at follow-up, this was 1.1% (4/373).

Because of the low case finding rate, the question was also asked, "How many of the parents had probable FH?" Fourteen of the parents tested had a TC and/or LDL-C above the 97.5th percentile for sex, and of these parents, seven had a significant family history of premature coronary heart disease (CHD), with a parent or grandparent having had an infarct under 65 years of age. Three of these parents had one or more such relatives affected in one generation, two had had both an affected parent and grandparent, and two fathers had had an infarct, and had both an affected father and grandfather (the child's great-grandfather). None of these parents had children who were HC at one year.

Dietary analysis. The mean daily dietary cholesterol intake was recorded as either a "low" cholesterol diet (0-100 mg cholesterol intake/day), a "moderate" cholesterol diet (101-250 mg cholesterol intake/day), or a "high" cholesterol diet (501-750 mg cholesterol intake/day). In retrospect, the 100 mg dietary cholesterol/day division may have been set too high, for included in that group was a sub-group of children whose intake was less than 50 mg/day, and another sub-group with an intake close to 100 mg/day.

At three months of age, 4% of the control group babies had a "low" cholesterol diet, and 16.5% had a "moderate" cholesterol diet. By 6 months, 59% had a "low" cholesterol diet, 38% a "moderate" cholesterol diet, and 2.5% a "medium" cholesterol diet. For the combined results of all the children reviewed at one year (groups 1-4 inclusive) the proportions had changed to "low" cholesterol diet 40%, "moderate" cholesterol diet 46%, and "medium" cholesterol diet

13%. No correlation was found between the serum cholesterol levels and dietary intake.

The possible influences of intra-partum stress factors on cord serum lipoproteins was investigated in those 52 babies whose cord serum LDL-C had been equal or greater than the 97.5th percentile of 1.97 mmol/L. This sub-group was aribtrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL-C would be evident in comparison with the control group. Among thse 52 HC neonates the cord serum TC and LDL-C was higher, though not significant on t-testing, for those with a birth weight less than 2500 gms, a gestation period of less than 37 or greater than 42 weeks, and those with a duration of labour greater than 11 hours. The mean values for cord TC and LDL-C were the same or lower, in comparison with those neonates who had not experienced these factors, in those whose labour had been induced, who had had any documented sign of fetal distress, whose one minute apgar score was less than eight, or who had required any resuscitative measure on delivery. No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies. Overall, the adverse factors occurred significantly in the 52 HC neonates than in the NC control group (Table 5:4), $p < 0.01$.

Discussion

This study was designed to test the hypotheses that an elevated cord serum LDL-C might be a marker for genetically determined HC, and that it might prove a more sensitive marker for future HC than cord serum TC alone, and to determine the proportion of HC infants who had had normal cord serum lipoprotein levels. Particular emphasis

TABLE 5:4 Percentage frequency of adverse fetal and perinatal events in control group children (n=382) compared with those who had a cord serum LDL-C equal to or greater than the 97.5th percentile (HC group) (n=52).

	Control Group	HC group
Birth weight <1.5 kg	3.4	34.6
Apgar 1 minute <6	12.6	38.5
Apgar 5 minutes <8	7.8	15.3
Mother aged <16	4.2	25
Gestation <37 weeks or <41 weeks	11.5	34.6
Any sign of intrapartum fetal distress	9.2	13.5
Baby needed resuscitation	9.2	23.1

was placed during the design of the study on ensuring a suitably large normal control population for prospective follow-up, a sequential analysis of LDL-C in as many cord serum samples as possible, and the use of cut-off points for cord blood TC and LDL-C derived from the 95th percentiles of the distribution curves of the actual sample.

Dominantly inherited FH can be reliably diagnosed clinically only with evidence of transmission through three generations of hyper-low density lipoproteinaemia, and/or the presence of tendon xanthoma. However, because of sociological factors in the sample reported here, it was only possible to obtain circumstantial evidence of three generation transmission from a history of premature coronary heart disease (CHD), and HC in parent and child.

In this study no differences were found at follow-up during infancy for the mean values of either TC or LDL-C between those children who had been HC or NC at birth. Of the six children with cord serum HC, and who were HC during infancy, only one (SM) had a parent with unequivocal HC. Another child, SB, had circumstantial evidence of FH with his father and brother having moderate HC (6.37 mmol/L), and the paternal grandfather having died from an infarct at age 55 years. SM's father had marked HC (7.8 mmol/L), with no history of early CHD. This high rate of false positive values, the minimum estimate being 3.7% may partly be explained by the influence of adverse perinatal factors which may cause an elevation in fetal serum cholesterol and TG levels (Cress et al, 1977; Potter, 1977; Chapter 6). Although the incidence of adverse factors such as short gestation, low birth weight, and signs of intrapartum stress were lower than in those with a cord serum LDL-C greater than the 97.5th percentile, amongst the latter group there

were no differences in the mean values for TC, LDL-C, or TG, in those who had or had not experienced such an event. The results therefore suggest that the individual perinatal experience of a child needs to be considered when assessing his/her cord serum lipoprotein level.

The results of this study also showed that not only did those children who had been HC as neonates have similar serum cholesterol values to those who had been NC as neonates when followed during infancy, but that transient HC was a common finding during the first year. Amongst those in the control group, altogether 7.4% were HC at 3, 6, or 12 months of age. This tendency to revert towards a normal serum cholesterol level was also described by Darmady et al (1972) in her study. All of her 22 HC one year olds had lower levels after a two month interval. None of the parents of these control group children who became HC during infancy had the characteristics of FH, with only four of these parents having moderate HC with a TC between 6.25 and 6.5 mmol/L. Two of these parents were young and obese fathers with mixed hypertriglyceridaemia and hypercholesterolaemia, the other two parents being healthy with no family history of early CHD. However, there was strong circumstantial evidence of FH in the families of two NC one-year-olds on the basis of paternal HC and early infarct, and experience of premature CHD in the generations, and in a further two families on the basis of parental HC, and CHD through two generations.

Although the attendance rate of children was far from complete, the control group was considered to be sufficiently large for a clear picture to emerge of the pattern of variation of lipid levels throughout infancy, and for detecting those children with false

negative results. The size of the control population was similar to the study ofDarmady et al (1972) but in both the large series reported from Cincinatti (Glueck et al, 1971; Tsang et al, 1974a), this aspect was not fully explored. In their studies, the control group comprised only 42 originally normo-cholesterolaemic children from a sample population of 1800. This control group was supplemented by a selected group of 60 ethnically different infants, whose serum cord lipids had not been measured. In a subsequent report of 3000 children form a different socio-ethnic population to the first study (Tsang, et al 1975), no control group was studied in parallel with the apparently hypercholesterolaemic babies. In the study of Greten et al (1973) a small group of 4% of the total sample population (65 of 1323) were seen at follow up, perhaps too small a number to clarify the incidence of false negative cord blood results. Andersen and Friis-Hansen (1976a)had nearly complete follow-up of their total sample population of 303 babies, but did not elaborate on the possibility of false negative results in those several babies whose total cholesterol at follow-up lay above the conventionally accepted upper limit of normal of 5.95 mmol/L (Fredrickson and Breslow, 1973).

During the first few months of life, the dietary cholesterol load may be an important influence on the serum cholesterol level (Fredrickson and Breslow, 1973; Friedman and Goldberg, 1975), with individual variation occurring between infants in the maturity of their homeostatic mechanisms (Chapter 9), so that children with FH may be NC on a low cholesterol diet (Tsang et al, 1974a). Indirect evidence for the emergence of a more mature homeostatic state with regard to cholesterol regulation comes from further data from the

study of the sample into their second year, with evidence for tracking for lipoproteins emerging and positive associations then appearing between the parents' and children's lipoprotein fractions (Chapters 7,8). Environmental factors thus may partly obscure the genetic influences on lipoproteins at least through infancy and prevent either the level of TC or LDL-C in cord blood, of during the first few months of life, being predictive for future HC.

Low density lipoprotein-cholesterol might be expected to act as a more accurate marker for subsequent hyperbetalipoproteinaemia than serum total cholesterol alone (Kwiterovitch et al, 1973). The mean values from this study and others for cord serum TC and LDL-C (or non HDL-C) and its percentage contribution to TC were compared; the value for TC from this study fell within the middle of the range of values from previous studies. Both the mean values for LDL-C and its percentage contribution to TC (and hence by inference the contribution of HDL-C to TC) were comparable to all the other previous studies except that of Andersen and Nielsen (1976). Measurement of LDL-C was used in the prospective series of Greten et al (1973), and pre-beta + betalipoprotein was used by Andersen and Friis-Hansen (1976). Their mean value for betalipoprotein was 0.91 mmol/l, with 1.56 mmol/l being their >2 S.D. cut-off point. This mean was the same as for this study (0.90 $\pm$ 0.47 mmol/l) and similar to that of Kwiterovitch (1970) of 0.93 $\pm$ 0.13 mmol/l on a series of only 36 babies. A calculated "non-HDL-C" value of 094 $\pm$ 0.53 mmol/l was reported by Glueck et al (1971) from 596 samples of the original 1800. However, they used Kwiterovitch's (1970) mean + 2 S.D. cut-off of 1.09 mmol/l, whereas that derived from their own data would have been 1.98 mmol/l, i.e. closer to the cut-off for this study.

This lower "borrowed" cut-off point for LDL-C may have significantly altered the interpretation of the results from the necessarily proportionately larger "hypercholesterolaemic" group of neonates.

The findings of this study are therefore in agreement with those of Darmady et al (1972) and of Potter and Nestel (1976b) who both found cord HC unreliable as a marker for future HC, and of Goldstein et al (1974) who approached the problem from an epidemiological angle, but have also shown that cord LDL-C does not improve the validity of cord-blood screening. The authors of other prospective studies including those of Glueck et al (1971), Tsang et al (1975), Greten et al (1973), and Andersen and Friis-Hansen (1976a) have concluded that children with FH may be detected by cord screening. Although this may be the case for individual families (Lee et al, 1969: Lewis et al 1967: Wolff 1967), and for children of parents affected with FH (Kwiterovitch 1973), it does not necessarily validate population cord-screening programmes. The information gained from this study has provided useful information as to the pattern and extent of individual variability of cholesterol levels during infancy, and has partly answered the doubts concerning the possible rates of false negative and false positive values in this screening procedure.

CHAPTER 6

FETAL, MATERNAL, AND INTRAPARTUM FACTORS AND THEIR EFFECTS ON CORD SERUM CHOLESTEROL AND TRIGLYCERIDE

INTRODUCTION

Both genetic and environmental influences determine an infant's lipoprotein profile. The relative importance of these influences may change: children with known familial hypercholesterolaemia (FH), having an elevated cord serum cholesterol, may be subsquently normo-cholesterolaemic (NC) on a low cholesterol diet (Tsang et al 1974a). If such genetic influences can be counteracted or mimicked through nutritional factors, to what extent can the intra-uterine environment alter the fetal lipoprotein pattern?

In a study of 1926 cord serum samples (Chapter 5), 3.7% had an elevated (95th percentile) cord TC and/or low density lipoprotein cholesterol (LDL-C) level, but were subsequently NC through infancy, i.e. false positive results. Events affecting fetal health in pregnancy and during delivery have been shown to relate to cord serum hyperlipoproteinaemia (Tsang et al 1974b, Andersen and Friis-Hansen 1976b, Andersen and Friis-Hansen 1977, Christensen 1977, Cress et al 1977, Potter 1977), with intra-uterine growth retardation and intra-partum stress being associated with elevated cord triglyceride levels, though unanimous results have not been found with regard to their effect on cord serum cholesterol. This retrospective analysis was therefore done to determine if maternal and perinatal factors known to be related to fetal stress were also related to elevations in cord serum cholesterol.

SAMPLE AND METHODS

Those children selected for a retrospective analysis of the maternal, fetal, and intra-partum factors relating to cord serum lipoprotein levels were considered in four groups:

Group 1. Those who had had a cord serum TC and/or LDL-C elevated above the 95th percentile (n=54). Their mean values were for cord serum TC 3.07 $\pm$ 0.3, and TG 0.41 $\pm$ 0.09 mmol/l.

Group 2. Those with both an elevated cord serum TG, and TC or LDL-C. The mean values were for cord TC 3.95 $\pm$ 1.78, TG 0.95 $\pm$ 0.18 mmol/l (n=8).

Group 3. Those with isolated cord serum hypertriglyceridaemia (HTg) above the 95th percentile, the TC being below that percentile (n=70). The mean values were for cord serum TC 1.85 $\pm$ 0.44, TG 0.85 $\pm$ 0.22 mmol/l.

Group 4. Those children whose cord serum TC and TG had been below the 95th percentile. The mean values were for TC 1.79 $\pm$ 0.52 mmol/l, TG 0.35 $\pm$ 0.16 mmol/l. (n=296).

The perinatal experience of each child was studied retrospectively from their case records. Information recorded included birth weight, gestational age, sex, age of mother, duration of labour, and the second stage, the apgar scores at one and five minutes, the method of onset of labour and delivery, and adverse maternal and fetal events. The categories used for each of these variables are included in Table 6:1.

Table 6:1 Categories of maternal and infant factors with mean ± SD values for cord serum total cholesterol (TC) and triglyceride (TG) mmol/L.

	n	Category	TC	TG
			Mean ± S.D.	Mean ± S.D.
Birth weight (kg)	34	<2.5	2.49 ± 1.06	0.66 ± 0.35
	266	>2.5	2.03 ± 0.73	0.47 ± 0.25
	212	>2.9	2.02 ± 0.74	0.46 ± 0.25
Gestation (weeks)	52	<37	2.39 ± 0.91	0.56 ± 0.30
	235	37 - 41	2.00 ± 0.74	0.47 ± 0.26
	249	>37	2.01 ± 0.74	0.48 ± 0.27
	14	>41	2.10 ± 0.87	0.66 ± 0.37
Apgar score at 1 minute	146	9 or 10	2.10 ± 0.82	0.44 ± 0.25
	82	7 or 8	2.14 ± 0.82	0.45 ± 0.21
	228	>7	2.11 ± 0.79	0.44 ± 0.24
	34	5 or 6	1.97 ± 0.62	0.62 ± 0.26
	23	3 or 4	1.88 ± 0.68	0.62 ± 0.37
	13	1 or 2	2.13 ± 0.77	0.83 ± 0.34
Apgar score at 5 minutes	151	9 or 10	2.04 ± 0.86	0.45 ± 0.21
	39	7 or 8	2.07 ± 0.67	0.58 ± 0.32
	190	<7	2.05 ± 0.82	0.47 ± 0.24
	8	5 o4 6	2.08 ± 0.68	0.97 ± 0.35
	3	3 or 4	2.02 ± 0.89	0.80 ± 0.10

Table 6:1 Contd:

	n	Category	TC	TG
			Mean ± S.D.	Mean ± S.D.
Age of mother (years)	3	<17	3.02 ± 0.53	0.40 ± 0.08
	55	17 to 21	2.15 ± 0.78	0.45 ± 0.25
	95	22 to 25	2.01 ± 0.76	0.53 ± 0.30
	93	26 to 29	2.18 ± 0.86	0.50 ± 0.28
	29	30 to 33	1.95 ± 0.67	0.38 ± 0.15
	17	34 to 37	2.65 ± 0.72	0.55 ± 0.33
	7	>37	1.74 ± 0.41	0.59 ± 0.22
Fetal events	205	Normal	2.15 ± 0.84	0.46 ± 0.25
	4	Tachycardia	1.92 ± 0.54	0.70 ± 0.29
	15	Bradycardia	1.73 ± 0.32	0.57 ± 0.36
	24	Meconium stained liquor	2.18 ± 0.74	0.43 ± 0.21
	7	Resuscitation + intubation	2.47 ± 0.97	0.65 ± 0.27
	19	More than one of above	1.74 ± 0.47	0.83 ± 0.32
	91	Any sign of distress	1.93 ± 23.8	0.56 ± 27.5

Two methods of analysis were used to determine whether factors related to possible perinatal stress were associated with an increase in cord serum TG and/or cholesterol levels. Firstly determining whether the mean values for cord serum TG and TC were significantly different on t-testing for those children with low birth weight, short gestation, documented signs of intra-partum distress etc., as defined in the categories in Table 6:1, compared to babies who did not have these factors. Secondly, determining whether the frequency of these events was higher in those who had elevated cord lipid levels compared to the normo-cholesterolaemic (NC) and normotriglyceridaemic (NTg) babies.

RESULTS

The relationship between fetal, maternal, and intra-partum events, and the cord serum TC and TG: the mean values for the cord TC and TG of each category used in the subsequent analyses are summarised in Table 6:1.

Babies of less than 2500 gm birth weight had significantly higher TG levels than those with a birth weight over 2500 gm and 2900 gm ($p<0.001$). They also had a higher mean TC level, significant at the 0.1 level.

Preterm babies, under 37 weeks gestation, had a higher mean cord serum TG ($p<0.05$) and TC ($p<0.001$) than term babies, and post-mature (over 41 weeks gestation) babies also had a higher mean TG ($p<0.01$).

Significant inverse correlations were present on regression analysis between gestational age and cord TC ($r - 0.20$, $p<0.001$, $n=301$) and cord TG ($r - 0.13$, $p<0.02$, $n=301$).

Babies with a one minute apgar score of seven or more had a lower TG than those whose score was 5 or 6, or 1 or 2 ($p<0.001$) for both. Those whose five minute apgar score was 3 or 4 had higher levels than those whose score was greater than 7, and those with a score of 7 or 8 had higher mean levels than those with a score of 9 or 10 ($p<0.01$ for both).

The coefficient of correlation between TG and the apgar score at one and five minutes was 0.34 (n=296) and 0.30 (n=203) respectively, $p<0.001$. It was not significant for cord TC, nor were any differences present between these categories for TC.

The mean value for cord serum TG was elevated compared to the value for the control group in those babies who had had intrapartum tachycardia or bradycardia, or who had subsequently required intubation and resuscitation, but not for those with meconium stained liquor. The TG was also significantly elevated for those with any sign of fetal distress ($p<0.01$), or more than one sign ($p<0.001$), compared with the control group. However, for both these conditions the mean TC was lower than in the control group ($p<0.05$ for both), and only for the seven babies who had needed resuscitation with intubation was the mean TC higher.

The cord serum TC was lower in those babies born after some procedure such as artificial rupture of the membranes, with or without a syntocinon infusion, or an elective caesarian section, compared with those born after a spontaneous onset of labour. It was higher (2.51 v 2.20 mmol/l) only for those four born after an emergency caesarian section. The mean cord TG was also higher in those born after an uncomplicated labour compared with those who had experienced some procedure (v.s.) and was lowest in the four babies born by

elective section after spontaneous onset of labour (means of 0.35 v 0.57 mmol/l).

There were no differences in the mean values for cord TC or TG in those babies born to younger or older mothers (Table 6:1) but mothers who had had pre-eclampsia, essential hypertension, or who had had an antepartum haemorrhage, or prolonged rupture of the membranes, had babies with a higher mean TC compared to unaffected mothers (means of 2.30 v 1.97 mmol/l), but no difference occurred for the mean values of cord TG.

Different durations of labour were not associated with significant differences in mean values for cord TC or TG.

There was also evidence for differences in the frequencies of adverse perinatal factors in babies with cord serum HC and/or HTg (groups 1 to 3) compared with the control group (4).(Table 6:2).

Babies in Group 1 (HC only) had a significantly higher frequency of birth weight under 2500 gm (LBW) ($p<0.02$), and had a lower mean: 3128 ± 500 v 3329 ± 294 gm. They were also more frequently preterm (gestation less than 37 weeks), or postmature (greater than 41 weeks) $p<0.001$.

Babies in Group 2 (HC and HTg) were also more frequently of LBW, $p<0.05$, means of 2900 ± 1223 gm versus 3329 ± 294 gm in the control group. They were also more frequently preterm or postmature ($p<0.001$).

There were no differences in the duration of the first or second stage of labour, the age of the mother, the mode of onset of labour, and the apgar score at one minute compared to NC babies. Similarly, there were no significant differences in maternal or intrapartum factors, but apgar scores at five minutes were significantly lower ($p<0.01$) compared to NC babies.

TABLE 6:2 The percentage frequency of adverse maternal, fetal, and intrapartum events in babies in Groups 1 to 4.

	Group 1	Group 2	Group 3	Group 4
Birth weight less than 2500 gm	17.0	42.8	18.8	4.8
Gestation period less than 37 weeks	29.6	50.0	21.4	10.2
Gestation period greater than 41 weeks	7.4	0.0	10.0	4.1
Apgar score at 1 min less than 7:	16.6	25.0	64.3	16.2
Apgar score at 5 min less than 8:	23.5	37.5	46.0	15.2
Duration of 1st stage labour more than 10 hrs	31.3	28.6	19.6	22.9
Duration of 2nd stage labour more than 30 min	21.9	25.0	60.0	29.9
Pre-eclampsia	10.5	0	22.2	15.3
Essential hypertension	7.9	0	7.4	1.8
Ante-partum haemorrhage	18.4	0	3.7	14.5
Prolonged rupture of the membranes	5.3	0	0	0.3
Intra-partum tachycardia	1.9	0	2.9	1.0
Meconium stained liquor	11.3	0	5.9	7.5
Resuscitation with intubation required	3.8	0	2.9	0.68

Babies in Group 3 (HTg) were also more frequently of LBW than control group babies ($p<0.01$), mean 3117 $\pm$ 399 gm, and were also more frequently pre term or postmature ($p<0.01$). The frequencies of both a prolonged first stage of labour (greater than 10 hours) and a second stage of greater than 30 minutes were greater than for the control group ($p<0.05$ for both), and clinical signs of intra-partum stress were also more frequent ($p<0.01$). The frequencies of low apgar scores at one and five minutes were also greater than for the control group ($p<0.001$).

Comparing the control (NC) babies with those in Groups 1 and 3, LBW ($p<0.001$), being pre or post-term ($p<0.001$), and having a prolonged first ($p<0.02$) or second stage of labour ($p<0.05$) were all more frequent.

DISCUSSION

The results of this study provide evidence that events known to be associated with hypoxic fetal distress, such as low apgar scores, or with adverse intra-uterine factors, resulting in preterm delivery, influence the levels of cord serum cholesterol and tri-glyceride levels, and are in general agreement with previous reports which are summarised for comparison in Table 6:3. Although the data was collected retrospectively, the factors used in the analyses would not be subject to significant observer error and the gestation of each baby was assessed by a paediatrician. Subjective bias was avoided by coding the factors without knowledge as to which cord lipid group the subjects had been assigned, and a larger sample was used than in previous studies.

The cord serum TC was found to be elevated in LBW and preterm infants, in agreement with other studies (Andersen and Friis-Hansen 1977, Cress et al 1977). Although non-significantly lower levels

Table 6:3 A synopsis of factors associated with cord serum hypertriglyceridaemia, from seven studies, and with hypercholesterolaemia from this study.

Reference	Sample Number	Cord TG Mean ± SD (mmol/L)	cut-off or 95th %ile	PET or hyper-tension	Prol. labor	Prol. rupt. memb.	Emerg. caes. sect.	Signs fetal dist.	Low Apgar score	Pre-term	Post mature	Small for dates	Low birth weight
Tsang et al (1974b)	102	0.41±0.20	0.84	+	+	+	-	+	+		+	+	+
Andersen & Friis-Hansen (1976b)	139	0.43	0.79	-	-	-	-	+	+	-	-	+	-
Cress et al (1977)	275	0.37±0.29	0.78	-	+	-	-	+	-	+			
Christensen (1977)	99	0.52		+	+			+ for SFD				lower	lower
Andersen & Friis-Hansen (1977)	91	0.40	0.89							+		+	
Potter (1977)	219	0.60±0.16		+	+			+	+			+	
This study	428	0.38±0.16	1.53	-	-	-	-	+	+	+	+		+
		Cord TC 1.83±0.56	2.86	+	+	+	+	-	-	+	-		+

+ = factor associated with elevation
- = no effect

were found in babies who had experienced intrapartum tachycardia and bradycardia in contrast to the raised levels found by Cress et al (1977), those babies who required resuscitation with assisted ventilation had elevated levels. The higher frequency of raised cholesterol levels found with maternal hypertension, pre-eclampsia, APH or after prolonged rupture of the membranes may represent a second order effect of the fetal stress, or be a fortuitous association.

Elevated levels of cord serum TG were found to be associated with fetal intrapartum bradycardia, tachycardia, and resuscitation but not with meconium stained liquor. Low apgar scores, being pre or post term, or a low birth weight were also associated with HTg.

The association between intra-uterine growth retardation and cord hypertriglyceridaemia was not looked for specifically because of the way in which the data was coded in the case records. No association was found between cord HTg and maternal hypertension or pre-eclampsia, APH, or prolonged rupture of the membranes.

The presumptive mechanism for fetal HTg and HC in the wide range of associated conditions is probably through the final common pathway of hypoxia, with subsequent lipolysis (Andersen and Friis-Hansen 1976b). Although there is normally at birth a marked release of catecholamines (Lagercrantz and Bistoletto 1973) which is exaggerated if asphyxia or an abnormal presentation occurs, or if the baby is pre or post term, or small-for-dates (Cheek et al 1963, Holden et al 1972) there is conflicting evidence as to the metabolic response in these babies (Harris 1974, Keele et al 1966). Harris (1974) found that the normal postnatal rise in free-fatty acids (FFA) was less marked in both normally grown and small-for-dates

hypoxic neonates, while lower peak levels during the first twelve hours after delivery were described for babies with mothers with PET and diabetes, or with delayed onset of respirations (Keele et al 1966). These studies on small samples and with wide variations of FFA levels do not invalidate the theory of an increased hepatic production of very low density lipoprotein secondary to an increase in FFA flux from peripheral tissues, causing the observable elevated TG levels in cord sera of stressed babies.

This study shows that an adverse intrapartum experience may produce marked changes in fetal lipid metabolism, counteracting genetic influences on basal cholesterol levels and invalidating the future predictive value of cord sera cholesterol estimation.

CHAPTER 7

FAMILIAL, NUTRITIONAL, AND ANTHROPOMETRIC CORRELATES OF SERUM CHOLESTEROL

INTRODUCTION

Although the differences in serum cholesterol levels between populations (Whyte and Yee 1958) are mainly due to nutritional factors, clear differences in serum cholesterol and triglyceride also occur between vegetarians and people on a mixed diet containing animal protein in the same population (Sacks et al 1975, Simons et al 1978), and certainly an infant's serum cholesterol can be significantly altered by major alterations in dietary fat, and cholesterol composition (Sweeney 1961; Chapter 9). Differences in activity level, body fat, and genetic factors may contribute lesser influences between individuals.

However, despite this evidence for the importance of dietary factors in cholesterol regulation, within an homogenous sample of healthy families these effects may be blurred and no apparent correlation between diet and serum cholesterol may occur (Hitchcock and Gracey 1977, Weidman et al 1978). This study was therefore designed to determine both the extent of familial, nutritional, and anthropometric factors on the level of cholesterol in early childhood, whether these influences change in relative importance during the first two years of life, and to investigate whether any significant correlates of serum cholesterol levels were present among anthropometric and nutritional factors measured in older children.

SAMPLE

The sample comprised those children followed to two years, and the schools sample. Although amongst the babies there was a significant decline in the numbers seen during the study period,

for the following regression analyses all the variables chosen had to be present for a child's case to be included in the calculation. Hence the actual numbers forming the samples on which the analyses were performed (as shown in the tables) were smaller than the actual number of children seen because of some missing data element at one or another point in time.

The analyses used were simple regression and hierarchical inclusion regression in which the computer expresses the variables in rank order of their influences on the dependent variables selected, in this case serum cholesterol, as assessed by the significance of the correlation coefficient. As the independent variables are added to the list, the multiple r value and its significance is given.

The breast-fed babies' data could not be included because values for their daily mean food energy intakes were not known. Hence, the numbers used in the analyses for three months, when approximately 50% of babies were being breast fed, were rather lower than the actual total sample seen.

From the questionnaire concerning the family experience of vascular disease, a coronary event below age 65 was taken as a significant family history of coronary disease (FHCD), and the family histories were graded from 'high risk' (a coronary event through two or more consecutive generations e.g. paternal great-grandfather to paternal grandfather) to 'no risk' (no history of any of the listed disorders) as set out in Table 7:1 set 3. Although this variable was only at an interval level, rather than at an ordinal level, as was the case for the others used, it was considered a valid method of introducing this important factor into the analyses. This variable was used in the babies' analyses.

TABLE 7:1. Set 1, 2, and 3, of variables included in the hierarchical inclusion regression analysis.

SET 1	Nutritional variables	
	Mean daily intake of protein (gm)	
	carbohydrate (gm) (cho)	
	fat (gm)	
	cholesterol (mg) (chol)	
SET 2	Nutritional variables	
	% body mass as lean body mass	(LBM)
	Sum of four skinfold thicknesses	(SFT)
	Serum total cholesterol of mother	(TCM)
	father	(TCF)
SET 3	Family history of early CHD (FHCD) graded from:	
	a. High risk: angina and/or infarct under 65 years in two consecutive generations	
	b) Moderate risk: as above; one or more events, only in one generation.	
	c) Low risk: no infarct or angina, but occurrence of hypertension, stroke and/or diabetes, P.V.D., HC, in one or more generations.	
	d) No risk: no history of any of the above conditions TCF and TCM	

RESULTS

The correlation coefficient of the parents' and children's cholesterol levels from birth to two years showed a significant association appearing at age two for TC for both mothers and fathers (r 0.25, $p<0.001$). During infancy the association was marginally more significant for the mothers' than for the fathers' TC, but for the fathers the association for LDL-C became significant at one year (r 0.27, $p<0.005$) and for the mothers at two years (r 0.23, $p<0.005$) Table 7:2. There was no association for either parent for HDL.

With the analyses repeated for boys' and girls' TC separately, interesting differences appeared, with the correlations being stronger overall between the boys and their mothers, and for the girls and their fathers (Table 7:3).

For the boys' LDL-C (Table7:4) a significant association emerged at one year, and strengthened to two years when it was again greater for the mothers (r 0.45, $p<0.001$) than for the fathers (r 0.39, $p<0.002$). No significant association occurred between the girls and either parent. For HDL-C there was a weak association only between the levels in cord serum and the parents' levels.

These regression analyses were repeated using apoB instead of cholesterol for the selected sub-sample of the 84 two year olds, and 82 mothers and the 38 fathers on whom the measures had been done (See Chapter 4).

The correlation between apoB of the children at two years with that of the parents is shown in Table 7:5. A combined analysis of the boys' and girls' data showed that there was a more highly significant correlation between children and their fathers (r 0.50) than for

TABLE 7:2. Coefficients of correlation between total serum cholesterol LDL and HDL in parent and child

		Birth	(n)	3	(n)	6	(n)	12	(n)	24 mths	(n)
TC	Fathers	0.12 n.s.	(76)	0.18†	(125)	0.18†	(125)	n.s.		0.25**	(113)
	Mothers	0.2†	(120)	0.13†	(190)	0.14†	(193)	0.17*	(189)	0.25***	(180)
LDL	Fathers	0.18 n.s.	(57)					0.27*	(96)	0.24***	(92)
	Mothers	n.s.						0.17†	(156)	0.23***	(162)
HDL	Fathers	0.15 n.s.	(71)					n.s.		n.s.	
	Mothers	0.2†	(118)					n.s.		n.s.	

† p <0.05 ** p <0.005

* p <0.01 *** p <0.001

TABLE 7:3. Coefficients of correlation between total serum cholesterol levels in parent and child.

	Birth	(n)	3 mths	(n)	6 mths	(n)	12 mths	(n)	24 mths	(n)
MOTHERS:										
Boys	0.32*	(60)	0.19†	(92)	0.27**	(94)	0.27**	(92)	0.35***	(90)
Girls	n.s.		0.15 n.s.	(95)	n.s.		n.s.		0.10 n.s.	(87)
FATHERS:										
Boys	0.16 n.s.	(39)	0.12 n.s.	(95)	0.22†	(62)	n.s.		0.28†	(59)
Girls	n.s.		0.28	(61)	0.14 n.s.	(61)	n.s.		0.25†	(52)

† $p < 0.05$ ** $p < 0.005$

* $p < 0.01$ *** $p < 0.001$

TABLE 7:4. Coefficients of correlation between LDL and HDL cholesterol of child and parent.

		Birth	(n)	1 year	(n)	2 years	(n)
MOTHERS:							
Boys	LDL-C	0.16 n.s.	(46)	0.24†	(78)	0.45***	(83)
	HDL-C	0.27†	(57)	n.s.		n.s.	
Girls	LDL-C	-0.14 n.s.	(49)	n.s.		n.s.	
	HDL-C	0.15 n.s.	(59)	n.s.		n.s.	
FATHERS:							
Boys	LDL-C	0.26 n.s.	(28)	0.34*	(50)	0.39**	(50)
	HDL-C	0.28 n.s.	(34)	n.s.		n.s.	
Girls	LDL	0.12 n.s.	(29)	0.20 n.s.	(46)	n.s.	
	HDL	n.s.		0.11 n.s.	(45)	n.s.	

† $p<0.05$ * $p<0.01$ ** $p<0.002$ *** $p<0.001$

TABLE 7:5. Coefficient of correlation between ApoB level in parent and child at two years according to sex.

	(n)	Father	Mother
Girls	17	0.40*	0.75**
Boys	67	0.52**	0.18 n.s.
Boys & Girls	87	0.50**	0.34*

* p <0.05

** p <0.001

TABLE 7:6. Coefficient of correlation between ApoB level in parent and child at two years, controlling for either the mother or the father.

		Control for ApoB of			
	(n)	Mother		(n)	Father
Boys : Father	21	0.40*	Boys : Mother	21	n.s.
Girls : Father	14	0.25 n.s.	Girls : Mother	14	0.71**

* p <0.05

** p <0.001

n.s. not significant.

children and their mothers (r 0.34). The level of apoB in girls correlated more highly with that of the mothers (r 0.75) whilst the apoB of the boys correlated more strongly with the fathers' level (r 0.52).

The coefficient of correlation between apoB level of parent and child at two years was also calculated controlling for either the mother or the father. The correlation between the girls and fathers and between the boys and mothers decreased in significance while that between the girls and their mothers remaining similar (Table 7:6).

For the hierarchical inclusion regression the following three data sets were used as independent variables (from Table 7:1) set 1, the nutritional variables alone; set 2, the nutritional variables, the fathers' TC (TCF), the mothers' TC (TCM), SFT and LBM ; set 3, TCF, TCM, and FHCD. The results of these analyses for ages three, six, twelve, and twenty four months are presented in Table 7:7 for sets 1, 2 and 3, with the children's TC used as the dependent variable. Using HDL-C and LDL-C as the dependent variables the results for sets 1 and 2 are presented in Table 7:8.

For set 1, the nutritional variables, there was a low degree of correlation at each age studied for TC, HDL-C and LDL-C. None of these variables were consistently selected first. When these analyses were repeated, including the daily energy intake per kg body weight no differences in the degree of correlation occurred.

For set 2, the nutritional, anthropometric, and parents' cholesterol values, the overall multiple r value increased through to two years (0.41, $p<0.005$), being greater for the boys (0.55, $p<0.05$) than for the girls (0.36, n.s.). The order in which the variables

TABLE 7:7. Results of hierarchical regression analysis with serum total cholesterol as the dependent variable for sets 1, 2, and 3 at three, six, 12 and 24 months The first two variables selected in order, the number of cases (n), and the multiple r value is given with its significance when $p<0.05$.

	3 months			6 months			12 months			24 months		
		n	r		n	r		n	r		n	r
Set 1												
All cases	fat chol	226	0.10	prot cho	220	0.14	cho chol	264	0.07	prot chol	191	0.13
Boys	fat protein	106	0.14	prot	108	0.14	cho protein	132	0.15	fat	97	0.13
Girls	chol protein	118	0.12	chol protein	109	0.14	F-level insufficient			cho fat	90	0.15
Set 2												
All cases	TCF fat	72	0.30	LBM TCM	80	0.34	LBM TCM	115	0.24	TCM protein	115	0.41**
Boys	TCM SFT	35	0.35	TCM TCF	37	0.45	TCM protein	59	0.44	TCM SFT	59	0.55**
Girls	TCF TCM	35	0.45	fat SFT	40	0.37	LBM fat	53	0.31	TCF protein	53	0.36
Set 3												
All cases	TCM TCF	48	0.30	TCM FHCD	49	0.28	TCM TCF	48	0.18	TCF FHCD	47	0.26
Boys	TCM FHCD	22	0.47	TCM FHCD	23	0.51	TCM TCF	23	0.58*	TCM FHCD	22	0.53
Girls	FHCD TCF	26	0.26	FHCD TCF	26	0.36	F-level insufficient			TCF TCM	25	0.24

* $p < 0.05$

** $p < 0.005$

TABLE 7:8. Results of hierarchical regression analysis with high (HDL-C) and low (LDL-C) density lipoprotein cholesterol as the dependent variables for sets 1 and 2, at one and two years. The first two variables selected in order, the number of cases (n), and the multiple r value is given with its significance when $p<0.05$.

Dependent variable Age (years)	HDL-C One			HDL-C Two			LDL-C One			LDL-C Two		
		n	r		n	r		n	r		n	r
Set 1:												
All cases	fat cho	247	0.12	cho protein	187	0.05	cho protein	240	0.18	protein chol	185	0.18
Boys	prot cho	128	0.19	fat protein	95	0.18	cho protein	124	0.21	chol protein	93	0.22
Girls	chol cho	115	0.17	fat protein	88	0.21	chol cho	112	0.19	cho fat	88	0.20
Set 2:												
All cases	TCF fat	108	0.36	TCF chol	111	0.15	TCF fat	105	0.28	TCF protein	109	0.40*
Boys	TCF fat	57	0.52	TCF LBM	57	0.34	TCF cho	54	0.48	TCM TCF	55	0.63**
Girls	LBM cho	48	0.33	TCM protein	51	0.36	LBM protein	48	0.44	protein cho	51	0.35

* $p < 0.05$ ** $p < 0.01$

were selected changed with time, there also being differences in order of selection between boys and girls. For the boys, TCM was consistently the strongest correlate, whereas for the girls TCF was the strongest at three months and two years of age. Addition analyses using height, body mass, SFT, and LBM, were no more significant than the set 2 variables at one and two years of age.

With HDL-C as the dependent variable in set 2, there was a stronger correlation than with TC for the boys at one year (r 0.52); but with LDL-C as the dependent variable, the correlation was stronger at one year than for TC for both boys (r 0.48) and girls (r 0.44) but at two years for the boys only (r 0.63, $p<0.01$) Table 7:8.

For TC as the dependent variable in set 3, the boys' correlation coefficient was maximal at age one year (r 0.58, $p<0.05$) with TCM followed by FHCD being consistently selected at each age apart from one year. For the girls, the association was weaker, reaching 0.36 at six months, and 0.24 at two years, with no consistent order of selection of the three variables (Table 7:7).

The results for the regression analyses for serum cholesterol on nutritional and anthropometric parameters for boys and girls each arbitrarily divided into three age groups corresponding to early, mid, and late puberty have been summarised in Tables 7:9 and 7:10. For daily energy intake expressed as mJ per Kg body weight and intake of protein, fat, carbohydrate, and cholesterol, no consistent correlation emerged for any variable for either sex.

The anthropometric variables used comprised height, weight, fatness as measured by skinfold thickness, lean body mass, and physical work capacity (PWCM). Again the level and direction of association varied between age groups and between the sexes, though

TABLE 7:9. Nutritional and anthropometric correlates of serum cholesterol levels. Correlation coefficient (when >0.1) and significance given when p <0.05; otherwise recorded as not significant (ns).

	mJ/kg	prot	fat	cho	chol	ht	wt	SFT	LBM	PWCM
	a. Boys under 12.5 years (n=49) except for PWCM where age was 11.5 to 12.5 years (n=6)									
TC					-	-	-	-	-.1	-.24
LDL-C			.13	-	-	-	-	-	-.15	-.31
HDL-C		-.27+	ns	.13	-	-	-	-.25+		.66
TG	-.24	.32+	ns	-	.20	-	-	-	-	-.92++
	b. Boys from 12.5 to 14.4 years (n=28) except for PWCM where age 13.5 to 14.4 years (n=11).									
TC	.18	-	-	-	-	-.35+	-.14	.16	-.25	-
LDL-C	.24	-.15	-	-	-	-.36+	-.21	-	-.27	-20
HDL-C	-.15	-	-	-.10	-	-	.11	.35+	-	-.52+
TG	-	.18	-.19	-	-	.19	.11	-	.13	.16
	c. Boys from 14.5 years (n=39) except for PWCM where age 14.4 to 16.5 (n=22).									
TC	-.15	.10	.22	-.21	-	-	-	-	-	.14
LDL-C	-	-	.21	-.19	-	-	-	-.25	-	.19
HDL-C	-	-	-.12	-.11	-	-	-	.30+	-.16	-.10
TG	-.20	.17	.27+	-.27	-	.26	.41	.21	.39++	-

\+ p <0.05

++ p <0.01

TABLE 7:10. Nutritional and anthropometric correlates of serum cholesterol levels. Correlation coefficient (when >0.1) and significance given when p <0.05; otherwise recorded as not significant (ns)

	mJ/kg	prot	fat	cho	chol	ht	wt	SFT	LBM	PWCM
	a. Girls under 11.5 years (n=39) except for PWCM where age was 9.5 to 11.5 (n=22)									
TC	-	-	-	-	.27^{x}	-	-	.26^{x}	-.21	.17
LDL-C	-	-	-	-	.31	-	-	.24	-.11	-.15
HDL-C	.31^{x}	-	-	-	-	-.61^{xxx}	-.49^{xxx}	-.29^{x}	-.46^{xxx}	-
TG	-.56^{xxx}	-	-	-	-.29^{x}	.34^{x}	.53	.59^{xxx}	.23	-.14
	b. Girls from 11.5 to 14.4 years (n=35) except for PWCM where age was 12.5 to 13.5 (n= 7)									
TC	-.22	-	.10	-.10	.30^{x}	.44^{xx}	.19	-	.25	.67^{x}
LDL-C	-.25	.22	.16	-.19	.29^{x}	.25	.13	.14	-	.51
HDL-C	-	-.23	-	-	.18	.39^{x}	-	-.22	.23	.66^{x}
TG	-.21	-	-.13	-	-	.38^{x}	.36^{x}	.11	.44^{xx}	-.11
	c. Girls from 14.4 years (n=39) except for PWCM where age was 14.5 to 16.5 years (n=22)									
TC	-	-.29	-.1	.20	-.14	.16	-	-	-	.36
LDL-C	-.17	-.21	-	.14	-.14	.13	.13	.13	-	.41^{x}
HDL-C	.21	-	.11	-	.23	-	.36	-.32^{x}	-.13	-.13
TG	-	-.21	-.28	-.28^{x}	-.18	-	.18	.39^{xx}	-.25	.21

x p <0.05

xx p <0.01

xxx p <0.001

a negative correlation occurred for HDL-C and these variables in the younger and older girls.

DISCUSSION

A child's serum cholesterol level increases rapidly in the post natal period, continuing to rise until towards the end of the first year, then declining to two years (Chapter 4). Major dietary changes are taking place during this period, and this study was designed to investigate the relative effects of genetic and nutritional influences, and their possible changes.

The results show clearly that positive associations between parents' and childrens' serum cholesterol levels emerge during the second year of life, and that sex differences become evident with these associations being stronger for the boys than for the girls, and especially between the mothers and their sons. This suggests that familial influences become stronger from the first year. The results from the multiple regression analyses supported this, both with evidence that, for the boys especially, the parents' TC was an important associated factor for TC, LDL-C, and HDL-C, but that the correlation for each of the nutritional variables used was low throughout the time-period studied.

These regression analyses allow an estimate to be made of the interdependence of two variables, in this case the serum cholesterol levels of parent and child, and this provides a measure of its heritability. Although few studies have used this method, Godfrey et al (1972) reported significant associations for cholesterol levels between parents and their children; Mimura (1975) found a correlation between parent and child of 0.26, and between sibs of 0.28; Valkenburg et al (1977) found the correlation to be higher for between child and mother (0.36) than between child and father (0.22). These

differences in sex-correlations were similar to the findings of this study for TC, and also to those of Shaeffer et al (1958) who reported correlations of 0.20 for father and child, and 0.36 for mother and child. However, although their correlations were higher for between father:daughter (0.26) than father:son (0.16), those for mother:daughter (0.39) were higher than for mother:son (0.34). However, the analyses performed on the apoB component of LDL show that the correlation between two-year-old children and their mother was much lower and less significant than that between the children and their father. The separate analysis done for boys and girls also showed sex differences with the correlation between the apoB of girls and fathers being only just significant whereas that between boys and mothers not reaching significance. However, the apoB levels in the boys correlated very highly with that of the fathers, whereas that of the girls correlated with the mothers' value. These sex differences also suggest a genetic component in the inheritance of apoB. A larger study on fresh serum samples would have to be done to verify the finding. There are no other similar studies with which these findings can be compared and it is proposed that this technique may be useful in the investigation of familial influences on the serum cholesterol level in children.

Major qualitative differences in intake of dietary fat are causally associated with differences in serum cholesterol levels both for sub-populations (Ostwald and Gebre-Medhin 1978, Räsänen et al 1978), as well as for individual adults and babies (Von Lossonczy et al 1978). The total daily intake of dietary fat is itself much less important in facilitating these changes than differences in the relative composition from saturated versus polyunsaturated fatty acids, with dietary cholesterol intake alone being a major

determinant (Hegsted et al 1965). Although large individual differences occur in dietary cholesterol handling capacity, an increase in neutral sterol and bile acid excretion while on a polyunsaturated fatty acid intake largely accounts for these changes (Nestel et al 1975). Despite this evidence, community studies on children do not produce results which would corroborate these findings (Hitchcock and Gracey 1974). Several explanations may account for this apparent discrepancy (Berwick 1978): the variation between diets in a homogenous sample of healthy children such as described in this study is rather small; errors in estimation of food intake in children at home are unavoidable, but may be compounded; and fluctuations of individuals' serum cholesterol may introduce a confounding factor of greater magnitude than the amount of real dietary induced differences in serum cholesterol levels.

The occurrence of the family history of early CHD as an influential variable is of interest, and raises questions as to the significance of differences in serum cholesterol levels within the observably normal range in relation to CHD. A separate analysis on these children has shown that those with a history of early CHD through two consecutive generations had a higher mean TC level than those children with no history of CHD in any antecedent relative (Chapter 11).

Measurements of body stature, size, fatness, and fitness failed to show any meaningfully significant association either in the two-year-olds or in the pubertal children. It is probable that if these factors do have a small effect on cholesterol levels, then it would be several stages removed from any clinically measurable parameter, or at least be obscured by other confounding variables in the regression analyses. Only with much larger samples over a

wider range of size might trends emerge.

In conclusion, this study shows that, within a sample of healthy children followed through to two years of age, there was no evidence that nutritional factors strongly influenced serum cholesterol levels, but that familial factors became clearly influential during the second year of life. The question of whether the emergence of the importance of these factors also reflected maturation of the child's homeostatic control for serum cholesterol is discussed in Chapter 8. Anthropometric factors were not shown to be influential either, and it is probable that the method of analysis used was too coarse and the sample sizes too small to detect true differences due to these factors.

CHAPTER 8

TRACKING OF SERUM CHOLESTEROL

INTRODUCTION

The serum total cholesterol (TC) concentration of children rises rapidly from a mean of 1.73 mmol/l at birth to 4.3 mmol/l at three months of age, after which it continues to rise slowly during the first year, but subsequently decreases until the early school years from which it again slowly rises to early adulthood (Chapter 4). However, by mid-adult life up to one third of the population may be defined as HC (Lewis et al 1974a, Slack et al 1977). Which of those HC adults were HC as children, or could have been identified as being on the track of HC? Since there is evidence that atherosclerosis begins in childhood (Mitchell 1973), with a strong association between HC and the incidence of CHD (Lewis et al 1974b), the control of HC is an essential part of long term primary prevention of CHD (Karvonen 1974, Mitchell 1973). It is therefore important to determine the relative importance of the genetic and environmental factors which influence the serum level of cholesterol in childhood (Editorial 1977a), and perhaps to identify those children who carry excess risk of CHD due to HC.

The concept of tracking of a biological variable, describing how its relative position on the frequency distribution curve is maintained through time, may be used as an index of the timing of an onset of a child's homeostatic process. With the evidence for changes in the relative importance of nutritional and genetic influences on cholesterol metabolism in early childhood (Chapter 7), this study was designed to determine when tracking emerged for cholesterol.

SAMPLE AND METHODS

The children studied, and the methods of cholesterol analysis are described in Chapters 2 and 4.

Simple two-way regression analysis of the cholesterol level at time one on the level at time two was used to assess the degree of tracking of this variable. Scatter of the values through time would give a low value; maintaining a ranked order with time would give a higher value.

RESULTS

The relationship between cord serum total cholesterol (TC) and subsequent serum levels as measured by regression analysis was weak at three (r 0.20) and six months (r 0.18), but became stronger at one (r 0.29) and two years (r 0.32). However, there was a marked difference in this association between the boys and girls with the relationship becoming stronger for the boys at one year (0.32) and for girls at two years (0.46) with the overall correlation being higher for the girls (Table 1).

The values of the association for LDL-C and HDL-C between birth and one year were 0.25 and 0.31 respectively, both values being lower between birth and two years (Table 8:1).

For the whole sample there was a gradual strengthening of the association between TC levels from three to six months (r 0.34), to one to two years (r 0.40). However, this change was more evident in the boys from whom the r value increased from 0.25 to 0.40; in the girls the value remained fairly constant between 0.40 and 0.45 (Table 8:2).

For LDL-C between one and two years the association was stronger for the boys (0.41) than for the girls (0.26) (Table 8:2) but for HDL-C the strength of the relationship was not significant for either sex.

TABLE 8:1. The relationship (r value) between serum total cholesterol (TC), low density (LDL-C) and high density (HDL-C) lipoprotein cholesterol levels at birth and at intervals through to two years (n).

		3 months r		6 months r		12 months r		24 months r	
TC	Combined	0.20**	(252)	0.18*	(229)	0.29**	(204)	0.32**	(119)
	Boys	0.06^{ns}	(127)	0.16^{ns}	(115)	0.32**	(103)	0.19^{ns}	(60)
	Girls	0.35**	(125)	0.21†	(114)	0.27*	(101)	0.46**	(59)
LDL-C	Combined					0.25*	(163)	0.18†	(103)
	Boys					0.21^{ns}	(83)	0.11^{ns}	(52)
	Girls					0.29*	(80)	0.20^{ns}	(51)
HDL-C	Combined					0.31**	(150)	n.s.	
	Boys					0.29†	(78)	n.s.	
	Girls					0.35*	(72)	n.s.	

** $p < 0.001$ * $p < 0.005$ † $p < 0.05$

TABLE 8:2. The relationship between levels of cholesterol at intervals through the first two years.

	Months	3 to 6		6 to 12		12 to 24	
TC	Combined	0.34**	(297)	0.38**	(274)	0.40**	(188)
	Boys	0.25*	(151)	0.30**	(140)	0.40	(96)
	Girls	o.45**	(146)	0.43**	(134)	0.40	(92)
LDL-C	Combined					0.35**	(163)
	Boys					0.41**	
	Girls					0.27	

** $p < 0.001$

* $p < 0.005$

DISCUSSION

The distance percentile chart for height provides the most commonplace example of tracking, in which a biological parameter retains its relative position through time. Does this phenomenon apply to non-somatic biochemical measurements, such as serum cholesterol?

With the increasing awareness that risk factors for CHD may have their origins in early life (Kannel 1976b), it may be of importance to know whether adults with an unequivocably abnormal serum cholesterol level (or diastolic blood pressure) could have been identified before the level became obviously abnormal. Conversely are those children in the top deciles for TC destined to become frankly HC in early adult life, and if so, at what age could they be identified?

The results of these analyses show convincing evidence that tracking for cholesterol emerges during the first two years of life, with interesting sex differences being present. Regression analyses of the variable at time one on time two allow the strength of the degree of tracking to be measured. Separate analyses on these children's height measurements gave an r value of 0.85, corresponding to similar longitudinal data but from child to adulthood (Tanner et al 1956), and allowing a comparison between the strength of tracking of a variable under almost entirely genetic influence (height) with a biochemical measurement (serum cholesterol) influenced both by nutritional (Reiser 1973, Truswell 1978), and genetic factors (Heiberg 1974). The square of the r value gives an approximate measurement of the degree of interdependence of the variables (Herden 1954) so for height this would be about 80%, and for TC

between one and two years 16%.

The interesting change in the strength of the association between the cord serum cholesterol level and the levels through to two years with the correlation being strongest at one year for boys and at two years for girls suggests that during infancy other (probably nutritional) factors blur the effect of the genetic influences which emerge later (Chapter 7), and which mediate to a large extent the cord serum levels of lipoproteins, despite local environment influences (Cress et al 1977, Chapter 6).

There is little other reported information about tracking of cholesterol in childhood. Ranking for TC was noted in the Busselton study (Godfrey et al 1972), and those who were HC tended to remain HC in the Tecumseh study (Epstein 1977), though in the Muscatine study (Lauer et al 1977) two thirds of the HC children reverted towards the mean. Since tracking of cholesterol implies maturity of its homeostatic mechanisms, one might predict that familial patterns of HC would become more apparent from age two. The validity of screening for HC from one year through to teenage has been shown to improve from age two, with an increasing proportion of HC children having HC parents (Chapter10).

In conclusion, the data reported here provides evidence of biochemical programming for serum cholesterol becoming established by two years of age, and suggests that, as the effect of the various influences becomes stabilised, it may allow predictions to be made about a child's likelihood of becoming HC in later life.

CHAPTER 9

INFANTS RESPONSE TO CHANGES IN DIETARY CHOLESTEROL AND FAT

Introduction

The serum cholesterol concentration at birth is low in all mammals that have been studied. The levels rise rapidly during the suckling period but decline substantially at the time of weaning in those animals that consume little fat (Carroll and Huff, 1977). Infants that are fed artificial milk mixtures containing little cholesterol show lower serum cholesterol levels than those fed human or cow's milk (Friedman and Goldberg, 1975), although the values remain appreciably higher than at birth. While the rise in the serum cholesterol in infancy may therefore be only partly explicable by changes in the intake of cholesterol and fats, it is possible that the response to these dietary factors is different in infancy to that shown by older children or adults. It may be that the low correlation between the cholesterol levels at birth and at one year of age, despite the presence of tracking for cholesterol observed in later childhood (Godfrey et al, 1972; Chapter 8), reflects the submersion of genetic by dietary influences in early life.

Some of the metabolic factors, which are probably genetically determined, that maintain cholesterol homeostasis in the body when fats and cholesterol are eaten, include changes in cholesterol synthesis and in cholesterol and bile acid excretion (Quintao et al, 1971; Nestel and Poyser, 1976; Connor et al, 1964; Nestel et al, 1975). These compensatory mechanisms have been found to operate in infancy (Potter and Nestel, 1976c) although it is not known whether the efficiency of these processes changes during the first year of life, nor is it known whether the cholesterol-raising

effects of diets rich in animal fats and cholesterol differ in infants from those seen in adults.

The differences in the serum cholesterol concentration and in the sterol balance in response to two diets that might respectively raise or lower the cholesterol level were measured during the first 16 months.

Sample and Methods

Ten infants were studied, four between the third and fifth months of life and six between the eleventh and sixteenth months as shown in Table 9:1. They were selected from the main study group on the basis of their mother's interest and likely cooperation. The studies consisted of two dietary periods each of about five weeks' duration based on a soy bean oil "milk" formula and the other based on cow's milk. The four younger infants consumed very little further fat from solid foods, the additional food items being derived from cereals, fruits and vegetables. The six older infants consumed virtually no cholesterol during the soy bean "milk" period, their solid foods consisting of cereals, fruits and vegetables. During the cow's milk diet period additional animal fat and cholesterol was provided as eggs and as measured amounts of meat, cheese, and butter. The diets of the infants prior to the study was similar to that for the second study diet.

Since studies of this duration cannot be reasonably carried out in a metabolic ward, a special attempt was made to obtain reliable measurements of food intake and sterol excretion that were supervised by the mothers.

TABLE 9:1 The composition of the two test diet (a and b): cholesterol content, proportion of calories as fat and ratio of polyunsaturated to saturated fatty accids in the whole diet.

Infant		Age (mth)	Wt (kg)	Cholesterol (mg)	Dietary Intake Fat %	P/S	Milk Formula*
1	a	4	6.9	0	33	3.0	Prosobee
	b	5	7.5	11	39	0.7	Similac
2	a	3	5.2	0	45	4.0	Procobee
	b	4	6.0	116	50	0.1	Cow
3	a	3	6.2	0	46	4.0	Prosobee
	b	4	6.8	93	48	0.1	Cow
4	a	3	6.0	0	45	4.0	Prosobee
	b	4	6.3	78	52	0.1	Cow
5	a	13	10.8	0	33	4.3	Prosobee
	b	14	11.4	380	42	0.3	Cow
6	a	14	12.5	0	29	3.3	Prosobee
	b	15	12.9	240	30	0.1	Cow
7	a	11	8.0	0	34	4.2	Prosobee
	b	12	8.6	300	38	0.2	Cow
8	a	15	10.6	0	40	4.6	Prosobee
	b	16	10.7	469	48	0.1	Cow
9	a	14	12.2	0	35	0.9	Isomil
	b	15	12.3	170	40	0.1	Cow
10	a	10	10.8	0	33	4.0	Prosobee
	b	11	11.3	165	38	0.1	Cow

* Source of milk. Prosobee: soy bean "milk"; Similac and Isomil contain more polyunsaturated fatty acids than found in cow's milk but not as much as in Prosobee.

The mothers were instructed about weighing the food, and about keeping a daily diary of food actually consumed. The items of food were restricted to those that could be readily measured and subsequently analyzed for fatty acid composition and cholesterol content with precision. These items were milk, cheese, butter and eggs. Other fat containing foods such as meats were provided as canned products and were also chemically analyzed. This technique of limiting allowable food items coupled with the provision of these items in preweighed packages has been used with success in studies of older children (Nestel, Martin, and Court - unpublished data).

An analysis of the daily food diaries and the chemical estimations of aliquots of foods were used to compute the fat and cholesterol intake.

The commencement of the 8-day fecal collection was monitored by administering carmine red during the fourth week of each dietary period. All the children were in nappies, and the mothers used disposable liners to catch the feces which were then frozen in their home deep freezers.

The infants were weighed regularly and gained similar amounts of weight during both periods.

A fasting sample of blood was obtained at the end of each dietary period for the cholesterol content of serum. The total 8-day collection of feces was pooled, homogenized and analysed in duplicate for cholesterol, bile acids and their steroid derivatives by gas chromatography (Nestel and Poyser, 1976).

Results

Serum Cholesterol

The serum cholesterol levels at the end of the two dietary periods are shown in Table 9:2). All infants had higher values during the cow's milk-cholesterol containing diet. In order to compare the responses in the younger and older infants the equations derived from adult studies* by Hegsted et al (1965) for the effects of dietary cholesterol and the saturation of the dietary fatty acids were used. In three of the four younger infants the observed difference in the serum cholesterol was close to or less than the calculated difference (Table 9:2). The fourth (Subject 3) showed a much higher than predicted increase with cow's milk. Of the six older infants, four showed a similar or a substantially smaller than predicted rise with the saturated fat-cholesterol diet. By contrast the remaining two (subjects 6 and 10, the latter known to have familial hypercholesterol-aemia) showed considerably greater than expected rises. For the group as a whole, the mean observed and "predicted" differences were almost identical, 1.46 and 1.51 mmol/L respectively, though there were few individuals in whom this was so.

* The equation that describes changes in the serum cholesterol to both changes in the intake of cholesterol and in the saturation of fatty acids is:

$$\Delta\text{serum cholesterol (mg/100ml)} = 2.16S-1.65P+6.77C-0.53$$

where S and P are the changes in the saturated and polyunsaturated fatty acids in the diet expressed as percentage of calories, and C is the change in cholesterol intake in 100 mg per day.

TABLE 9:2 Observed differences in the serum cholesterol concentrations during the two dietary periods

Infant	Serum cholesterol (mmol/L)			
	PUFA† period	Cow milk period	Observed Difference	Predicted Difference*
1	2.42	3.38	0.96	0.88
2	3.07	4.76	1.69	1.95
3	2.81	5.17	2.37	1.72
4	3.59	4.68	1.09	1.61
5	3.35	4.47	1.12	1.79
6	3.43	5.62	2.18	1.30
7	5.02	5.72	0.70	1.07
8	4.37	5.15	0.78	2.44
9	3.72	4.50	0.78	0.86
10	5.72	8.61	2.89	1.48
Mean Difference			1.46	1.51

† Polyunsaturated fatty acid-enriched milk formula

* Calculated from equations derived in adults for effects of saturation of dietary fatty acids and of cholesterol intake (Grundy and Ahrens, 1969)

Bile Acid Excretion

The sterol balance data is shown in Table 9:3. Bile acid (acidic steroid) excretion was substantially higher with the formula enriched with polyunsaturated fatty acids than with cow's milk in two of the four younger infants and in four of the six older infants. The very high excretion in subject 6 during the prosobee period may explain the much greater than predicted difference in the serum cholesterol between the two diets. A paired t-test analysis for the whole group of the differences between the amounts of bile acid excreted showed a significantly higher response to feeding the polyunsaturated fatty acids ($p<0.05$).

Net Sterol Balance

The other parameter of sterol balance which is shown in Table 9:3 is the net balance or the difference between total sterol output and the estimated intake of cholesterol. In the steady state, this value equals the amount of endogenously synthesized cholesterol though in rapidly growing infants this may only approximate cholesterol synthesis. The reduced values for net sterol balance in two of the four younger infants and in five of the six older infants implied a reduction in synthesis in response to the consumption of cholesterol. The marked increase in the serum cholesterol with cow's milk seen in subject 3 might have been due to failure to suppress cholesterol synthesis though a high serum cholesterol response to cow's milk was also seen in the hypercholesterolaemic infant (subject 10) despite apparent reduction in synthesis. Furthermore, subject 9, in whom cow's milk raised the serum cholesterol by only the predicted amount, also failed to show a reduction in the net sterol balance. A fall

TABLE 9:3 Responses to the two diets (a and b): Serum cholesterol concentrations and sterol balances

Infant		Serum Cholesterol (mmol/L)	Cholesterol Intake# (mg/d)	Bile Acid Excretion (mg/d)	Neutral Steroid Excretion (mg/d)	Net Sterol Balance (mg/d)
1	a	2.42	0	35(5.1)*	58	93(13.6)*
	b	3.38	11(2)*	15(2.0)	104	108(14.4)
2	a	3.07	0	26(5.0)	53	78(15.2)
	b	4.76	116(19)	8(1.3)	64	-44†(0)
3	a	2.81	0	27(4.4)	64	91(14.7)
	b	5.17	93(14)	36(5.3)	191	134(19.7)
4	a	3.58	0	11(1.8)	24	35(5.8)
	b	4.68	78(12)	11(1.7)	69	2(0.3)
5	a	3.35	0	47(4.4)	135	182(16.9)
	b	4.47	380(33)	53(4.6)	292	-35†(0)
6	a	3.43	0	139(11.1)	252	391(31.3)
	b	5.62	240(19)	47(3.6)	292	99(7.7)
7	a	5.02	0	58(7.3)	132	190(23.8)
	b	5.22	300(35)	23(2.7)	314	37(4.3)
8	a	4.36	0	72(6.7)	151	223(20.7)
	b	5.15	469(44)	21(2.0)	432	-16†(0)
9	a	3.72	0	55(4.5)	155	210(17.1)
	b	4.50	170(14)	13(1.1)	344	187(15.3)
10	a	5.12	0	31(2.9)	92	123(11.4)
	b	8.61	165(15)	30(2.7)	144	9(0.9)

* mg/kg/d in parenthesis

† minus net balance indicates retention of dietary cholesterol

estimated from daily food diaries and measurements of cholesterol content in aliquots of food items

in the net sterol balance might also reflect in part net retention of dietary cholesterol; this appeared to be the case in subjects 2, 5, and 8, though this did not lead to a greater than predicted rise in serum cholesterol levels.

Discussion

These studies are in agreement with previous observations that the serum cholesterol level is lowered when infants are fed foods low in cholesterol and in saturated fatty acids (Potter and Nestel, 1976c, Glueck et al, 1972). Comparing the changes in serum cholesterol of these 10 infants with the theoretical response that such a change in the intake of cholesterol and fat might produce in adults, (Hegsted et al, 1965) showed that seven of the 10 infants had changes in serum cholesterol that approximated or were less than the predicted values. Despite the limitations of this study such as the dependence on the mothers to provide the food, the single measurements of the serum cholesterol concnetration, and the growth of the infants, there was remarkable concordance between the average change in the serum cholesterol for all infants (1.46 mmol/L) and that reported in a large study in adults (1.51 mmol/L) for the corresponding change in dietary cholesterol and fatty acid composition (Hegsted et al, 1965). The present study therefore strongly suggests that the magnitude of the response to dietary cholesterol and fat becomes established in early life. The separate effects of cholesterol and of the type of fat cannot be identified from this study, and it is therefore uncertain whether the response to changing the cholesterol intake alone is as variable as reported in some studies in adults (Quintao et al, 1971; Whyte et al, 1977; Slater et al, 1976). In general, however, the

serum cholesterol is raised in adults, by increasing the saturated fatty acid and cholsterol intake, to levels that can be predicted at least for groups of individuals (Connor et al, 1964; Keys et al, 1965; Mattson et al, 1972). In this study both the fatty acid and the cholesterol intake were altered to determine the metabolic responses to more extreme manipulations of the serum cholesterol level.

The sterol balance data also revealed similarities to the metabolism of cholesterol and bile acids found in adults. The amounts of steroids excreted resembled the values for young children from Huang et al (1976), and from Carter et al (1975). Bile acid excretion was significantly higher with dietary polyunsaturated fatty acids which has also been demonstrated in at least normolipidemic adults (Connor et al, 1969; Nestel et al, 1975). One (subject 6) of three infants who showed a particularly large difference in the serum cholesterol between the two diets, excreted large amounts of acidic steroids with polyunsaturated fatty acids which might have accounted for the substantially lower serum cholesterol level on that diet; (this child's serum cholesterol at one year of age, prior to commencing the Prosobee period, was 5.20 mmol/L.

Reduced cholesterol synthesis is a well-documented response in adults to an increased intake of cholesterol (Quintao et al, 1971; Nestel and Poyser, 1976). Although the required steady-state in cholesterol metabolism can probably not be assumed in rapidly growing children, the marked fall in the net sterol balance when cholesterol was fed (seen in seven infants), would almost certainly have resulted partly from reduced synthesis. This was not observed in one infant

(subject 3) who showed an unusually large rise in the serum cholesterol with dietary cholesterol. Since the net sterol balance reflects changes in cholesterol reexcretion as well as in cholesterol synthesis, the findings in infants who failed to reduce their net sterol balance might have indicated an increase in excretion. This appears to be an additional mechanism that compensates for increased consumption of cholesterol, and it might have accounted partly for the unchanged or higher net sterol balance in two infants (subjects 3 and 9) one of whom showed the large rise in serum cholesterol. Reexcretion is a less effective compensating mechanism than suppression in synthesis and the apparent retention of cholesterol during cholesterol feeding that was found in three infants (subjects 2, 5 and 8) may have reflected failure to achieve a steady state though it did not lead to an unusual rise in the serum cholesterol.

The high serum cholesterol shown during the cow's milk, cholesterol-containing diet by subject 10, the only child with F.H., occurred despite suppression of cholesterol synthesis. Tsang et al, (1974a) have reported a significantly higher rise in serum cholesterol in hypercholesterolaemic than in normal infants on diets that included cow's milk and eggs.

In conclusion, of the three infants who showed unusually large differences in their serum cholesterol levels between the two diets, a large difference in bile acid excretion might have accounted for the finding in one (subject 6) and failure to suppress synthesis in another (subject 3). No apparent explanation can be provided in the third infant (subject 10).

CHAPTER 10

THE VALIDITY OF SCREENING FOR HYPERCHOLESTEROLAEMIA AT DIFFERENT AGES FROM 2 TO 17 YEARS

Introduction

With hypercholesterolaemia (HC) recognised as a potent risk factor in the development of CHD (Kannel, 1976b) several authors have suggested that paediatricians should be concerned about identifying those children carrying an excess risk due to HC. (Lloyd and Wolff, 1969; Williams and Wynder, 1976). Dominantly inherited familial hypercholesterolaemia (FH) is the commonest hereditable cause of HC, and carries an excess risk of CHD. Although identification of the condition may be possible at birth (Kwiterovitch et al, 1973) results from screening programmes have given conflicting results (see Chapter 5).

Although HC is common in childhood and adolescence (see Chapters 1 and 4) the actual serum level of a serum cholesterol sample which falls in the "grey zone" between 6.5 and 7.0 mmol/L is not diagnostic of FH, with both unaffected children having levels above and children with FH having levels below 6.77 mmol/L. Nonetheless, it may well be that screening procedures after infancy differ in accuracy according to the age group studied. Children of two, four (pre-school), and from eight to seventeen years were studied to see if there was an improved chance, with increasing age, of identifying those with an inherited form of HC.

The samples

The two year olds, four year olds and schools samples were used in this study. For the purposes of the study early CHD was defined as a parent or grandparent having evidence of ischaemic

heart disease under 65 years of age. HC in a parent was defined as a TC level above 6.5 mmol/L.

The parents of 16 of the 20 four-year-olds with HC underwent family lipid studies from two to 15 months after the initial sampling. Family studies on the school children's parents were completed within one month of the first sample.

Results

The two-year-olds. Fifteen of the 200 were HC (>95th percentile for TC and/or LDL-C) with eight having a level >97th percentile (Table 10:1). Four (cases 1,6,12,14) of the 15 had one or more HC parent of whom three had a history of early CHD. All but three (cases 1,5,10) of the 15 HC children had a family history of early CHD. Twenty-three of the 310 parents tested were HC, of whom fifteen had a history of early CHD.

The family history of each child was weighted according to whether the history of early CHD was in the parent, grandparent, or great-grandparent, and whether the parent appropriate to the side of the family with CHD was HC. On dividing the children into groups of those with a TC and/or LDL-C between the 95th and 97th percentiles, and those with one, or both values, above the 97th percentile, no difference was found for each group's average score. Thus the extent of the family's experience of CHD did not influence the degree of HC in the child.

The four-year-olds. Twenty pre-school children had a serum TC above the 95th percentile, with 9 having a family history of early CHD (Table 10:2). Four of these children (cases 21,22,25,27) had normocholesterolaemic parents and the other five had HC parents (cases 18,19,23,31,35). One of these children had a grandfather

TABLE 10:1. The family history of early CHD and TC value (mmol/l) of HC parents of the HC two-year-olds.

Sample No.	Family history	Parents' TC
1	Nil	Mo 6.71, Fa NC
2	M	Both NC
3	M & P	Both NC
4	M & P	Both NC
5	Nil	Mo NC, Fa NS
6	M	Mo 6.71, Fa NS
7	P	Mo NC, Fa NS
8	M & P	Mo NC, Fa NS
9	M & P	Mo NC, Fa NS
10	Nil	Both NC
11	P	Both NC
12	M & P	Mo NC, Fa 6.5 (with LDL >97th)
13	M	Both NC
14	M & P	Mo 7.05, Fa 7.23
15	M	Both NC

Legend:
P = paternal side of family
M = maternal side of family
Fa = father
Mo = mother
NS = not sampled
NC = normocholesterolaemic

TABLE 10:2. The family history of early CHD and TC value (mmol/l) of HC parents of the four-year-olds.

Case No.	Family history	Parents' TC
16*	Nil	Fa 8.22
17	Nil	NC
18	M	Fa 10.22
19*	P	Fa 7.98
20	Nil	Fa 7.70
21*	P	NC
22	M	NC
23*	P	Fa 8.22
24*	Nil	Fa 6.89
25	M	NC
26*	Not known	Adopted
27*	P	NC
28	Nil	NS
29*	Nil	NS
30*	Nil	NS
31	M	Mo 7.33
32*	Nil	NS
33	Nil	Mo 6.94
34*	Nil	Fa 7.77
35*	P	Fa 6.73

Legend:
P = paternal side of family
M = maternal side of family
Fa = father
Mo = mother
NS = not sampled
NC = normocholesterolaemic

* ≥ 97th percentile for TC or LDL-C

(case 19) and another a father (case 23) who had had a coronary bypass, and the mother of case 31 had xanthomata, her father having had an infarct at age 70. The father of case 35 had borderline HC (6.76 mmol/L) but a strong family history of early CHD and case 18 had an HC father, though her NC mother had the family history of early CHD.

Of the ten children with no family history of CHD five had an HC parent (cases 16,20,24,33,34), the parents of four children were not tested (cases 28,29,30,32) and one child was adopted (case 26).

If the 97th, rather than the 95th percentile value for TC had been used as a cut-off point, 12 children (cases marked with * on Table 10:2) would have been selected, of whom 5 had a family history of early CHD (cases 19,21,23,27,35) with 3 children (cases 19,22,35) having an HC parent, though the mother of case 31 with probable FH would have been missed. Among the seven children with no family history, three had HC fathers (cases 16, 24,34). Thus elevating the cut-off point would not have improved the case finding accuracy of the test.

On resampling the 20 HC children, five remained HC (cases 16,23,27,34,35). Of these children, four had an HC parent, two with probable FH (cases 23,35), two with no family history of CHD (cases 16,34), and case 27 had diabetes mellitus.

The primary school sample. The serum TC lay above the 95th percentile in four children of whom two (cases 36,38) had a positive family history of early CHD. The father of child 38 was moderately HC, and the father of child 36 was considered to have probable FH, having had an infarct at 42 years; his wife also has HC. Of these two children only child 38 had a TC above the 97th percentile, so if that level had been used as a cut-off point, child 36 would have been missed.

The secondary school sample. Seven students had a TC level above the 95th percentile, of whom five (whose TC levels were also above the 97th percentile) were available for family study (Table 10:3). There was a family history of early CHD in each family, but only the parents of three children (cases 41,42,44) were HC. However, only in the family of child 44 did the HC parent have the history of CHD on her side of the family.

Of the 235 school children interviewed, 79 (33.6%) had a positive family history of early CHD of whom 8 (3.4%) were HC. There was no difference between the values for TC, LDL-C, or high density lipoprotein cholesterol for those children with a family history of CHD (mean for TC 4.88 $\pm$ 0.94) compared with those children with no family history (mean for TC 4.96 $\pm$ 0.99 mmol/l).

DISCUSSION

The results of this study suggest that the case detection rate of children with probably genetically determined HC increases after the first two years of life. Although only three of the fifteen HC two-year-olds had an HC parent with a history of early CHD, twelve of the fifteen came from families with such a history. Similarly, fifteen of the twenty-three HC parents had a history of early CHD. Among the four-year-olds, half the parents of families either with or without a positive family history of early CHD were HC, and of the nine HC four-year-olds with a family history of early CHD, four (cases 19,23,31,35) children had parents with probable FH. A family history of early CHD was present for all those HC school children with an HC parent and although 33.6% of the school children had a positive family history of early CHD, only

TABLE 10:3. The family history of early CHD and TC value of the school sample.

Case No.	Family history	Parents' TC
	PRIMARY SCHOOL	
36*	Fa	Fa 5.47 (post infarct), Mo 7.23
37*	Not known	Adopted
38*	P	Fa 6.37
39	Not known	NS
	SECONDARY SCHOOL	
40*	M	NC
41*	P	Mo 6.71
42*	M	Fa 7.8
43*	M	NC
44*	M	Fa 6.89, Mo 6.81

Legend: P = paternal side of family
M = maternal side of family
Fa = father
Mo = mother
NS = not sampled
NC = normocholesterolaemic

* >97th percentile for TC or LDL-C

3.4% were HC when tested.

By selecting the 97th, rather than the 95th, percentile as a cut-off point, four of the eleven four-year-olds with an HC parent would have been missed, and the primary school girl (case 36) whose father had had an infarct at 42 years would not have been selected. The proportion of pre-school children with or without a positive family history would not have changed.

It was of interest that the majority of HC children detected showed fluctuations of their cholesterol level with time, with a tendency to revert to the normal range. This raises the question as to how many of those NC children with a positive family history might also be intermittently HC, and become HC adults.

With the present divergence of opinion as to if, when, and whom to screen for hypercholesterolaemia (Leonard et al 1976), it was considered of interest to ascertain if an optimal age for screening could be defined both in biochemical and practical terms.

The results suggest that the presence of a positive family history would enable a suitable sub-population to be selected out for further testing, although this would inevitably mean disregarding the possibility of detecting children from families susceptible to dietary-induced HC.

Although at the conclusion of the study the results were not clear cut, the results were useful in putting the problem into perspective, particularly when taken together with the previous results for the two-year-olds during their first year (Chapter 5).

The findings of this study are in agreement with those of Leonard et al (1976) who found a low case detection rate for FH when screening a sample of hospitalised children. The findings also suggest that cholesterol analyses on children with family histories of CHD would detect at least some people who carry an excess coronary risk, and that school entry could provide a suitable occasion for such a selected screening programme.

CHAPTER 11

FAMILY PATTERNS OF HEART DISEASE AND CHILDREN'S CHOLESTEROL

Coronary heart disease (CHD) runs in families (Editorial 1977b), and a history of an affected first degree relative constitutes a risk factor (Patterson and Slack 1974). Although in a small proportion of affected families the transmitted risk is related to familial hypercholesterolaemia (FH), it may be that lesser degrees of hypercholesterolaemia (HC) related to a combination of nutritional and polygenic influences may also contribute to the excess CHD risk which runs in some families.

This study was designed to test whether children with no significant family history of CHD had similar serum cholesterol levels to children who had positive family histories of early CHD. In Chapter 10 it was shown that HC children tended to have HC parents, especially from school age; but are lesser degrees of HC also transmitted, and if so, are they related to families' experience of CHD?

SAMPLE AND METHODS

The sample comprised those children followed to two years. Information concerning family history was obtained by questionnaire, followed by an interview with each mother. Many mothers had taken care to ask their parents about details of relatives' cause of death, so that information on the children's great-grandparents was validated. Full details were obtained on 160 cases, some missing information about grand or great-grandparents occurred in the others. From the family histories four grades of risk for CHD were arbitrarily defined:

Group 1	"no risk"	No reported occurrence of any disorder listed below (n=35)

Group 2 "no heart risk": No reported occurrence of angina or infarct, but stroke, hypertension, or peripheral vascular disease had been reported (n=222).

Group 3 "moderate heart risk": Reported occurrence of angina infarct under 65 years in one or more non-consecutive generation (n=105)

Group 4 "high risk": Angina or infarct reported in two or more consecutive generations, e.g. PGF, PGGF (n=29)

The mean $\pm$ values for the children's TC at each age studied and for LDL-C and HDL-C at one and two years in each group were calculated, and the differences between these means compared by T-testing.

The details of history and cholesterol levels in each family were scrutinised so that those parents and children with HC (TC>95th percentile: fathers>6.5, mothers>6.24, children aged 2>5.72 mmol/l) could be defined. The details concerning cholesterol analysis have been described in Chapter 4.

RESULTS

An upward gradient occurred for TC at each age studied from Group 1 ("no risk") to Group 4 ("high risk") Table 11:1. The differences were significant at three months between Groups 1 and 3 ($t = -2.41$, $p<0.01$), and Groups 1 and 4 ($t = -2.64$, $p<0.01$). At one and two years the significances of the differences were less,

TABLE 11:1. Mean values for serum total cholesterol (TC) mmol/L, between groups 1 to 4.

Serum TC at:	3	6	12	24 months
Risk Group	mean ± S.D. sample size	mean ± S.D. sample size	mean ± S.D. sample size	mean ± S.D. sample size
1	4.03 ± 0.62^{+*} 35	4.18 ± 0.86 35	4.25 ± 1.03^{e} 35	3.94 ± 0.87^{o†} 30
2	4.14 ± 0.85 222	4.37 ± 0.88 152	4.28 ± 0.83 118	3.95 ± 0.67 52
3	4.46 ± 0.98* 105	4.36 ± 0.92 102	4.55 ± 0.87^{e} 96	4.25 ± 0.72^{o} 86
4	4.51 ± 0.83^{+} 29	4.30 ± 1.03 29	4.54 ± 0.90 28	4.34 ± 0.63† 27

* $p < 0.01$

\+ $p < 0.01$

e $p < 0.05$

† $p < 0.05$

o $p < 0.05$

partly because of the smaller sample size. The differences between the groups' mean values was slightly greater for the girls than the boys at each age.

For LDL-C a similar trend occurred at both one and two years, of similar magnitude for boys and girls. Between Groups 1 and 3 at one year $t = -2.2$, $p<0.025$: and between Groups 1 and 4 $t = -2.16$, $p<0.025$, with a difference of similar significance at two years (Table 11:2).

The mean HDL-C level was significantly greater for Group 1 than for Group 3 ($t = 1.68$, $p<0.05$), or for Group 4 ($t = 2.1$, $p<0.025$), at one year only; at two years no significant differences were present between the groups' mean levels (Table11:2).The differences at one year were accounted for entirely by the boys in whom the mean level fell from 1.27 ± 0.51 in Group 1 to 0.90 ± 0.23 mmol/l in Group 4. No gradient occurred in the girls' HDL-C levels at either one or two years.

The serum cholesterol levels of the parents of the children in each group were also compared (Table 11:3). Despite the family histories referring only to the child, clear trends were present with those parents in Group 4 having higher levels than those in Group 1, with the gradient being greater for fathers' than mothers' TC and LDL-C. No differences occurred for HDL-C.

The differences between the children's mean lipid values could have been due either to a true upward shift for the TC levels of those in Group 4 or to the presence of only several very HC children causing an upward skew in the mean value.

At two years in Group 1 (n=35) there was one HC child, four HC parents, but none with FH. In Group 2 (n=222) there were

TABLE 11:2. Mean values for LDL-C and HDL-C between groups 1 to 4 at one and two years.

Risk Group	LDL-C		HDL-C	
	1 year	2 years	1 year	2 years
	mean ± S.D. n	mean ± S.D. n	mean ± S.D. n	mean ± S.D. n
1	2.17 ± 1.03*+ 33	1.97 ± 0.73° 28	1.18 ± 0.45*+ 33	1.23 ± 0.43 28
2	2.20 ± 0.85 107	1.99 ± 0.75 49	1.04 ± 0.41 113	1.25 ± 0.28 50
3	2.56 ± 0.81* 88	2.13 ± 0.64 84	1.04 ± 0.38* 89	1.43 ± 0.76 85
4	2.72 ± 0.77+ 23	2.42 ± 0.78° 27	0.96 ± 0.24+ 24	1.27 ± 0.24 27

* p <0.025

\+ p <0.025

o p <0.025

TABLE 11:3. Mean values for TC and LDL-C for the parents of children in groups 1 to 4.

Risk Group	Serum TC		LDL-C	
	Father	Mother	Father	Mother
	mean ± S.D. n	mean ± S.D. n	mean ± S.D. n	mean ± S.D. n
1	4.99 ± 1.12 20	4.59 ± 0.77 29	3.03 ± 1.09 19	2.69 ± 0.71 27
2	4.91 ± 1.05 37	4.66 ± 1.03 56	2.90 ± 1.12 35	2.62 ± 0.88 52
3	5.27 ± 1.32 56	4.69 ± 0.91 84	3.07 ± 0.83 44	2.64 ± 0.81 78
4	5.54 ± 1.38 14	4.69 ± 0.85 27	3.29 ± 1.08 12	2.78 ± 1.28 25

no HC children, five HC parents, none with FH. In Group 3 (n=105) there were two HC children of whom one had been persistently HC through infancy, though his parents were normocholesterolaemic (NC). Eleven parents were HC with two fathers' TC being above 7.8 mmol/l, and both possibly having FH. In Group 4 (n=29) no children were HC, five parents were HC, of whom two fathers had probable FH with marked HC and a characteristic family history. Thus there was not an over-representation of HC children in the groups which could account for the differences in cholesterol levels.

DISCUSSION

"Heart disease is a family affair" (Bauer 1977), with men in particular experiencing a much increased risk of early angina or infarct if they have a first degree relative affected with premature CHD (Deutscher et al 1970, Patterson and Slack 1974, Sholtz et al 1975). Whether this increased risk is transmitted by chance (Editorial 1977b), by environmental influences (Brunner et al 1971), or by polygenic (Carter 1974), or single gene effects (Schaefer et al 1958) is in dispute.

This study was designed to test whether differences in families' experience of CHD was reflected in the serum cholesterol levels of their children even as early as from three months to two years. Single gene effects result in a higher than expected incidence of HC in children of fathers who have had an infarct in early middle age (Tamir et al 1972, Gross and Caplan 1978), and this effect would skew their mean TC. In this study this factor was taken into consideration for those few children for whom there was evidence of parental FH. The results showed an upward trend for both TC and LDL-C, inverse for HDL-C, from those children in the

"no risk" to those in the "high risk" group. These data therefore suggest the presence of influences on the serum cholesterol levels with which the association for a family history of early CHD could be either causal; or reflect mediation of linkage of inherited CHD - proneness with HC; or that the HC is one aspect of several inherited characteristics for high CHD risk in those families.

These results are similar, but for a younger age group, to those in the Tromsø study (Førde and Thelle 1977), and of Blumenthal et al (1975) and Dzinzindki and Puzyrev (1976), and are parallel to other studies which have shown an over-representation of HC in children of fathers with early CHD (Schaefer et al 1958, Tamir et al 1972, Chase et al 1974).

Positive correlation of parents' and children's TC levels occurred for the two-year-olds in this study, as has been reported for other populations (Schaefer et al 1958, Godfrey et al 1972, Mimura 1975), and in studies which have shown that HC is more prevalent than expected in children of HC parents (Namboodiri et al 1975, Morrison et al 1978). In this study the percentages of the parents of children in the "moderate risk" and "high risk" groups were certainly greater than in the other two groups, but not the proportion of HC children.

Dispute exists (Carter 1974), as to whether the increased frequency of HC in first degree relatives of young probands with CHD (Koshechkin et al 1976, Førde and Thelle 1977), is due to a single gene effect (Schaefer et al 1958, Goldstein et al 1973), or polygenic influences (Patterson and Slack 1974). Studying the similarity of age of death between siblings with FH (Heiberg 1977), provided a genetic model for which the results suggested

that additional factors to the serum TC level (which did not correlate with age of death) may account for much variability in the clinical expression of the condition; but that these factors might be environmental (e.g. smoking) was suggested in a study of factors which reduced the usual discrepancy of 9 years for the age of death between men and women (Beaumont et al 1976).

Twin studies provide a unique genetic model to study CHD, for the risk factors and clinical expression were strongly concordant in the Swedish Twin Study (de Faire 1976, Lilfefors 1976). However the evidence is conflicting as to whether the concordance for serum lipid levels is due mainly to genetic or environmental influences (Rifkind et al 1968, Orr et al 1975, Christian et al 1976, Feinleib et al 1977). There is indirect evidence from studies of infants coronary arteries in Finland for genetic factors being of significance in early life (Personen et al 1975), and this has parallels in animal work (McGill and Mott 1974, Grewal and Purusholtamian 1978). Circumstantial evidence for the importance of genetic factors in CHD also comes from such diverse markers as HLA - haplotypes (Matthews 1975) and dermatoglyphics (Rashad et al 1978).

Whatever the relative importance of genetic or environmental influences are in the development of CHD, the results of this study provide evidence that there are differences between children's serum lipid levels which reflect their families experience of CHD, and may subsequently relate to differences in its prevalence in their generation.

CHAPTER 12

IDENTIFICATION OF CORONARY RISK FACTORS IN CHILDREN

INTRODUCTION

Coronary heart disease (CHD) has a long silent, latent period, (Mitchell et al 1972). Its origins in atherosclerosis may, in some situations, e.g.familial hypercholesterolaemia (FH), clearly begin in childhood (Lloyd and Wolff 1969), though generally its antecedents may not be so well defined (Kannel 1976b). Nonetheless, there may be children who could be identified as at-risk from the early onset and appearance of factors known to be associated with an increased risk of CHD morbidity in adult life (Williams and Wynder 1976).

Several of these wellknown risk factors, such as hypercholesterol -aemia (HC) (Fredrickson and Breslow 1973), or an elevated blood pressure (BP) (Beaglehole et al 1975; Editorial 1978a; Zinner et al 1971), may be under both genetic and environmental influences so that the age for optimal screening for an abnormality may need to be defined. However, the accumulating evidence for tracking of both BP (de Swiet et al 1976; Zinner et al 1975),and serum cholesterol (Godfrey et al, 1972; Lauer et al 1977) suggests that children at future risk may be identified before their levels of risk factors, for example BP or serum cholesterol, actually reach a recognisably pathological level.

This study was designed to determine the extent of clustering of risk factors occurring in children. The presence of several apparently unrelated factors occurring together, parallels other large studies mainly concerning the prevalence of major risk factors in childhood (Clarke et al 1970; Lauer et al 1975).

The sample and methods.

The children in the schools sample and the four-year-olds were the subjects for this study. Each of the school children had been asked to complete a detailed questionnaire with the parents' help before the day of the study and this included questions concerning the cause and age of death of the relatives. This was checked at the interview with each child. A history of a coronary event (infarct or angina) occurring before age 65 years in either parent, grand-, or great grand-parent, was recorded as a positive for family history of coronary vascular disease (FHCD). For the four-year-olds, children were selected who lay in the uppermost and lowermost deciles (10%), and in the middle range between the modal and mean values of the observed frequency distributions for each sex for systolic or diastolic blood pressure (BP), serum total cholesterol (TC) or low density lipoprotein cholesterol (LDL-C) and fatness as defined from the sum of the four skinfold thickness measurements (SFT). These cut-off points are summarised in table 12:1.

For the school children, those falling in the top 5% of the population for TC, systolic or diastolic blood pressure (BP), the sum of the four skinfold thicknesses (SFT), or the lowest 5% for PWCM (or the age-adjusted lowest decile for PWC150), and with a positive FHCD were selected as having a "high-risk" factor. Those in the top 10% were described as having a "low-risk" factor. The values for these measurements and the cut-off points used are summarised in Table 12:2.

Results

The four-year-olds. Inter-relationships between height, weight, blood pressure, measurements of fatness and serum lipoproteins.

TABLE 12:1 Cut-off points based on top, modal, and lowest deciles for B.P. (mmHg), skinfold thickness (SFT) mm, and total TC and low density lipoprotein cholesterol (LDL-C) mmol/L, used for selecting those with clustering of risk factors in the four-year-olds.

Risk factor		Upper range	Modal range	Lower range
B.P. Systolic	Boys	>105	90	}<75
	Girls	>100	90	
Diastolic	Boys	}>70	}60	<48
	Girls			<45
SFT	Boys	>33	19-21	<17
	Girls	>38	22-24	<19
TC	Boys	>5.85	4.08-4.73	<144 ?
	Girls	>5.72	4.16-4.65	<137 ?
LDL-C	Boys	>3.90	2.37-2.60	<54 ?
	Girls	>3.96	2.13-2.63	<58 ?

TABLE 12:2 The values for mean, S.D., and 90th and 95th percentile cut-off points used in selecting those school children with low-, and high-risk factors for total cholesterol (TC), mmol/L; systolic (SYS) and diastolic (DIA) BP (mmHg); the sum of the four skinfold thicknesses (SFT) mm; physical work capacity adjusted to body mass (PWCM), and at a heart rate of 150 (PWC150) according to age adjusted decile.

Risk-factor		mean	S.D.	90th cut-off	95th cut-off
TC	Boys	4.92	0.95	5.98	6.68
	Girls	4.95	0.96	6.16	6.68
SYS	Boys	116.2	16.1	135	146
	Girls	113.4	13.1	129	138
DIA	Boys	58.5	11.4	71	78
	Girls	60.3	11.8	73	78
SFT	Boys	39.6	19.6	64	83
	Girls	60.0	28.4	98	117.6
PWCM	Boys	10.39	2.5		9.8
	Girls	8.4	3.8		7.6
PWC150					
Boys	<13.5 yr	467.4	19.7	250	
	13.5-14.9 yr			400	
	>14.9 yr			490	
Girls	<12.5 yr	369.9	115.9	150	
	12.6-13.4 yr			250	
	>13.4			330	

For girls and boys there were significant positive associations between body size and both blood pressure and fatness. For boys' systolic blood pressures, significant positive correlations occurred for both weight (r 0.28; $p<.001$) and height r 0.19; $p<.01$), and with the sum of skinfold thickness (SFT) (r 0.15; $p<.05$), percentage body weight as fat (BWF) (r 0.14; $p<.05$). For diastolic blood pressure, significant correlations were present only between weight (r 0.26; $p<.001$), and height (r 0.23; $p<.001$). For girls the systolic blood pressure was positively correlated with weight (r 0.23; $p<.005$) but not for height, and for SFT (r 0.16; $p<.02$), and BWF (r 0.14; $p<.05$). For both sexes significant correlations ($p<.001$) were present between body weight and triceps skinfold thickness, SFT, and the BWF (Table 12:3).

Identification of risk factors

For systolic blood pressure, the mean $\pm$ SD was 111 mmHg; 30 children had a systolic pressure of 110 mmHg or greater. The mean $\pm$ 2 SD for diastolic pressure was 76 mmHg; 4 children lay at or above this level, and 9 had a level of 70 mmHg (the 98th percentile), or greater.

Nine children's TC lay above 6.5 mmol/l either initially, or on a repeat sample (done on those with a TC >6.24 mmol/l which was the 95th percentile). One of these children had diabetes, and normocholesterolaemic parents, and another had a father with probable familial hypercholesterolaemia.

Clustering of risk factors

For girls 2.1% (5), and for boys 0.8% (2), had clustering of high values with 5.3% of boys and 5.8% of girls with values coinciding in the mid-range, but none had clustering of

TABLE 12:3. Coefficients of correlations and significance values for inter-relationships between height (HT), weight (WT), systolic and diastolic blood pressure, and measurements of fatness: triceps skinfold thickness (TRIC), the sum of skinfold thicknesses (SFT), percentage body weight as adipose tissue (BWF).

		WT	HT	SFT	BWF
Systolic BP	Boys	0.28^{xxx}	0.19^{xx}	0.15^{x}	0.14^{x}
	Girls	0.20^{xx}	ns	0.16^{x}	0.14^{x}
Diastolic BP	Boys	0.26^{xxx}	0.23^{xxx}		
	Girls	0.23^{xxx}	ns		
TRIC	Boys	0.30^{xxx}			
	Girls	0.34^{xxx}			
SFT	Boys	0.42^{xxx}			
	Girls	0.48^{xxx}			
BWF	Boys	0.42^{xxx}			
	Girls	0.45^{xxx}			

xxx $p < 0.001$ xx $p < 0.01$ x $p < 0.05$

low values. (Table 12:4).

Tracking.

Eleven girls and 10 boys were chosen for further study by virtue of having fallen in either of the 'high' or 'mid' groups for clustering. Their blood pressure and skinfold thicknesses were recorded on re-examination from 11 to 15 months after their initial examination. Coefficients of correlation were derived for the diastolic blood pressure between the values for the first and second measurements (r 0.57; $p<.005$). This was greater than that for systolic (r 0.30; $p<0.1$), as might be expected from its inherent lability. The coefficients of correlation were significant for the sum of the skinfold thicknesses (r 0.75), the subscapular (r 0.92), and the triceps skinfold (r 0.42). (Table 12.5).

The school children. Of the 118 boys studied 12 (10.1%) had one high risk factor, of whom 9 had one or more additional low risk factors. Excluding those with low PWC, the numbers were nine (7.6%) and six respectively. For the 117 girls, 16 (13.7%) were in this category; excluding those with low PWC there were 11 (9.4%) of whom five had one or more additional low risk factors. These results are summarised in Table 12:6.

Two high risk factors were present in 28 (23.9%) boys and 26 (22.2%) girls. Fourteen boys and 17 girls had a low PWC and a FHCD with three boys and three girls each having an additional low risk factor; five boys and one girl had a low PWC and another high risk factor other than FHCD. Six (5.1%) boys and five girls had two high risk factors other than a low PWC.

TABLE 12:4. Clustering in upper, modal, and lowest deciles for blood pressure, serum cholesterol, and fatness, for boys (n) and girls (n).

Boys (244)			Girls (242		
High	Mid	Low	High	Mid	Low
0.8%	5.3%	0	2.1%	5.8%	0

TABLE 12:5. Coefficients of correlations (r), and significance (p) between triceps (TRIC), subscapular (SUB), and total (SFT) skinfold thickness measurements, and systolic (SYS) and diastolic (DIA) blood pressure recordings, from the initial to the follow-up examinations

	r	n
TRIC	0.42^{x}	21
SUB	0.92^{xx}	18
SFT	0.75^{xx}	21
SYS	0.30^{x}	20
DIA	0.57^{x}	19

xx $p < 0.001$

x $p < 0.01$

TABLE 12:6. The numbers of boys and girls with one to three high-risk factors, with and without additional low-risk factors present, and including and excluding those with low PWC.

Number of factors present	including those with low PWC only		excluding those with low PWC only		Total %
	without additional low-risk factors	*with* additional low-risk factors	*without* additional low-risk factors	*with* additional low-risk factors	
1. Boys		3	3	6	12 10.1%
Girls		5	6	5	16 13.7%
		8 : 3.4%	9 : 3.8%	11 : 4.7%	28 (11.9%)
2. Boys	19	3	6	0	28 23.9%
Girls	18	3	5	0	26 22.2%
	37 : 15.8%	6 : 2.6%	11 : 4.7%		54 (22.9%)
3. Boys	4	0	0	0	4 3.4%
Girls	5	0	0	0	5 4.3%
	9 : 3.8%	0	0	0	9 (3.8%)

Three high risk factors occurred in four boys and five girls, of whom four had an FHCD and a low PWC, with two having a high SFT and two a high BP. Two of the boys had an FHCD and a low PWC with one having HC and the other a high SFT. The other two had a low PWC and a high SFT and BP.

Overall, 54 (45.6%) boys and 60 (51.3%) girls had a positive FHCD, with 22.8% of boys and 26.5% of girls having this risk factor only present. Of the 27 boys with FHCD, 15 had a low PWC only in addition to FHCD, with three having a low PWC and one or more low risk factors. For the 29 such girls, the equivalent numbers were 17 and three.

Of the 44 boys and 41 girls selected as having a low PWC (not included in the Table), only 14 boys and 11 girls did not have an additional risk factor; it was other than a positive FHCD in six boys and one girl.

In Table 12:7 are presented the mean values for the variables in each category. There was an equal sex ratio, and the mean ages suggest an overall upward skew, but no upward shift in age for the group with most factors. The mean values for the other factors measured show an upward trend for TC, systolic BP, and diastolic BP, particularly for those with more concordant risk factors.

Discussion

Much recent interest had centred on the possibility and practicability of defining in early life those carrying an excess risk of CHD (Kannel and Dawber 1972). With significant differences in CHD mortality between ethnically similar populations (Olsson 1974), and the evidence that primary preventive measures, even in adult life,

TABLE 12:7. Values (mean ± S.D.) for each factor for the children in each category, as per Table 3

Number of factors present		Including those with low PWC only		Excluding those with low PWC only	
		Without additional low-risk factors	*With* additional low-risk factors	*Without* additional low-risk factors	*With* additional low-risk factors
1. Boys	N		3	3	6
	AGE		14.4 ± 1.8	11.9 ± 1.7	16.0 ± 1.0
	SYS		127.3 ± 14.7	123.3 ± 12.2	143.7 ± 20.7
	DIA		58.7 ± 13.3	54.0 ± 12.4	63.8 ± 14.3
	TC		4.92 ± 1.00	6.10 ± 2.40	5.44 ± 1.05
	SUMSF		48.6 ± 14.3	40.1 ± 17.1	38.7 ± 13.4
	PWCM		8.9 ± 0.5	10.9 ± 1.0	12.7 ± 2.0
	PWC150		548.3 ± 42.5	468.0 ± 174.1	824.6 ± 164.4
	FHCD		None	None	2 cases
Girls	N		5	6	5
	AGE		13.1 ± 1.8	14.8 ± 2.7	15.1 ± 2.5
	SYS		122.4 ± 13.2	122.7 ± 14.2	121.6 ± 13.3
	DIA		62.8 ± 5.8	62.7 ± 13.1	71.6 ± 4.8
	TC		5.40 ± 1.53	5.98 ± 1.28	5.74 ± 1.11
	SUMSF		33.9 ± 9.4	65.2 ± 40.1	88.6 ± 32.5
	PWCM		6.3 ± 2.8	8.2 ± 1.4	8.9 ± 0.74
	PWC150		257.0 ± 125.6	523.8 ± 158.4	510.0 ± 147.6
	FHCD		None	None	3 cases

TABLE 12:7. continued ....

2. Boys	N	19	3	6
	AGE	13.0 ± 2.6	14.1 ± 4.1	15.5 ± 2.1
	SYS	117.0 ± 12.3	125.3 ± 16.2	131.7 ± 18.6
	DIA	61.1 ± 12.6	68.0 ± 8.7	62 ± 18.3
	TC	4.64 ± 0.92	5.45 ± 0.98	6.09 ± 1.50
	SUMSF	38.7 ± 16.7	48.0 ± 20.3	66.6 ± 34.5
	PWCM	7.8 ± 1.7	8.1 ± 1.3	12.1 ± 1.4
	PWC150	359.2 ± 151.8	435.0 ± 49.5	797.0 ± 99.9
	FHCD	14 cases	2 cases	5 cases
Girls	N	18	3	5
	AGE	11.9 ± 2.7	13.6 ± 2.9	14.6 ± 2.7
	SYS	110.0 ± 10.6	122.0 ± 8.0	134.0 ± 7.4
	DIA	58.7 ± 8.7	72.0 ± 5.3	74.0 ± 9.8
	TC	5.01 ± 0.96	4.82 ± 0.72	6.20 ± 0.81
	SUMSF	61.7 ± 23.1	111.1 ± 29.0	80.9 ± 41.7
	PWCM	5.9 ± 1.4	5.1 ± 0.9	8.3 ± 3.8
	PWC150	263.9 ± 104.7	300.0 ± 26.4	473.3 ± 273.6
	FHCD	17 cases	2 cases	3 cases

TABLE 12:7. continued ....

3. Boys	N	4
	AGE	11.5 ± 3.4
	SYS	125 ± 22.7
	DIA	70.0 ± 21.1
	TC	5.45 ± 0.82
	SUMSF	79.5 ± 37.6
	PWCM	8.3 ± 0.69
	PWC150	493.8 ± 222.0
	FHCD	2 cases
Girls	N	5
	AGE	13.0 ± 3.1
	SYS	129.2 ± 17.0
	DIA	76.4 ± 19.0
	TC	4.76 @ 0.87
	SUMSF	106.4 ± 23.5
	PWCM	5.4 ± 1.7
	PWC150	315.0 ± 121.3
	FHCD	4 cases

may reduce its prevalence (Farquhar et al 1977; Goldberg et al 1975; Karvonen 1974), acquired and environmental factors in addition to family patterns of CHD-proneness have been implicated (Royal College of Physicians 1976).

In this study, the prevalence and rate of concurrence of risk-factors (clustering) was measured. These included the presence of a family history of an early coronary event (the overall incidence in the sample being nearly one half), being physically unfit, and fat, as well as being HC or having an elevated BP.

In the school sample there was a significant proportion of children who were HC, had a high BP, or were obese, or unfit, or had an FHCD, in some combination. Overall 8.5% had one major risk factor excluding a low PWC; 11 (4.7%) having two or these risk factors (excluding a low PWC), and 54 having two factors, including the large number, 31 (13.2%), with a low PWC and an FHCD. Nine (3.8%) had three concurrent risk factors. In the four-year-olds clustering of modal values occurred in similar proportions of boys and girls (5.3 and 5.8%) but high values in fewer boys (0.8%) than girls (2.1%), though no clustering occurred for the lowest values. Although it is not possible to discriminate between fortuitous association, genetic influence, or causal inter-relationships between these factors, these results do suggest that risk factors not only increase in prevalence with age, but that clustering also increases with age. The results are in line with, though have extend -ed the investigation of clustering, studies from Holland(Valkenburg et al 1977; Kromhout et al 1977), Denmark (Strunge and Trostman 1978), Austria (Markel and Rudas 1976), and from several large community

studies in the U.S.A. in which the prevalence of CHD risk factors in children has been measured (Wilmore and McNamara, 1974; Voors et al, 1976; Webber et al, 1977; Gilliam et al, 1977).

Although there is a well recognised association between frank obesity and an increased blood pressure both in children (Court et al, 1974a) and adults (Kannel et al, 1967b) the relationship between blood pressure and degrees of fatness in the normal range is less clear, particularly for preschool children. Lauer et al, (1976), in a group of older children, found overweight children over-represented in the upper percentiles for blood pressure, and significant correlations between systolic pressure and triceps skinfold thickness and weight were found in 8-year-olds (Stine et al, 1975). Thus the presence of significant inter-correlations between blood pressure and both weight and degree of fatness in the four-year-olds was of interest, for, allowing for the possible contribution to systolic pressure from an increased arm circumference in overweight children, it suggests that this well recognized relationship may be established at an early age and for children in the range of observed normality.

The lack of correlation between serum lipoproteins, and in particular triglyceride, and fatness at this age is of interest because of the known association in adults (Albrink and Meigs, 1964; Hollister et al, 1967) and in frankly obese children of 12 years or more (Court et al, 1974b). This may represent a threshold effect related to adipose size and subsequent insulin resistance (Olefsky, 1976). The serum cholesterol was not positively correlated with systolic blood pressure as had been described for older children (Florey et al, 1976).

Although the long-term significance of these risk factors in children cannot yet be clearly related to a subsequently increased risk of CHD, there is evidence for ranking of both BP (Heyden et al, 1969; Zinner et al, 1975) and serum cholesterol (Godfrey et al, 1972; Epstein, 1977) with time with those in the upper percentiles remaining there, as seen in this four-year-old sample. A positive family history of an early coronary event is itself a fixed risk factor (Patterson and Slack, 1974). A longitudinal study of PWC has also shown both that tracking occurs and that this dynamic measurement of health declines particularly in teenage girls (Bailey, 1973) corroborating the cross-sectional PWC data from this study (Chapter 3).

It is speculative as to the extent of genetic versus environmental influences in this situation, though there is evidence that clustering of risk factors in adults occurs more frequently than expected from chance alone (Morton et al, 1977).

In conclusion, these results suggest that realistic primary preventive measures against CHD need to be started in school age children with health-oriented programmes aimed at helping children learn about the benefits to long term health of exercise, good nutrition, and weight control.

CHAPTER 13

SUMMARY AND CONCLUSIONS

The results of these studies have provided information about the pattern of change of serum cholesterol through childhood, and the extent of the influences of the various factors which regulate the level. The relevance of hypercholesterolaemia (HC) to family patterns of risk for CHD was investigated in screening studies of HC from the newborn period to school age, and was extended to include screening for other CHD risk factors in the preschool and school age children.

The level of cholesterol in cord serum was found not to accurately predict which children would remain, or become, HC. The high proportion of false negative and false positive results invalidated its use as a screening procedure. Presumed hypoxic factors associated with perinatal stress were related to changes in cord serum cholesterol and triglyceride levels. These changes would increase the false positive and negative rates in a cord serum screening programme.

Although changes in dietary fat and cholesterol were associated with marked changes in the serum cholesterol of individual babies, analyses of the effects of nutritional factors in a large sample of normal children failed to show diet-related differences for serum cholesterol. By following children longitutinally over two years the changes in the degrees of correlation between the parents' and their children's cholesterol level could be studied. The increasing strength of the association during the second year, together with the evidence for an increasing level of tracking for serum cholesterol in the second year, was interpreted as evidence

that familial, presumably genetic, factors were becoming of increasing importance as influences on serum cholesterol after infancy. Further evidence for this was found in the results of the screening studies done at different ages, for from two years the proportion of HC children who had an HC parent and a significant family history of premature CHD increased. By secondary school age such a family history provided a discriminant factor in selecting those children likely to be HC, though not necessarily affected by familial hypercholesterolaemia (FH).

Evidence that familial CHD risk patterns (excluding FH) could be transmitted by differences in cholesterol level short of marked HC was found in the two year olds. Whether this trend for children from families with no history of CHD to have lower total serum cholesterol levels (but higher HDL cholesterol levels) than those with a significant history of premature CHD increases with age, and is itself causally related to differences in levels of atherosclerosis, is speculative.

The results of screening children for CHD risk factors such as HC, elevated BP, obesity, showed that the prevalence rate for concordance of these factors increased from early to later childhood. This data therefore suggested that environmental influences acting on constitutional factors might be causative in the emergence of a recognisable coronary risk profile in some teenagers.

These data therefore provide evidence that biochemical programming for the serum cholesterol concentration becomes established after the first year, and probably during the second year of life. Familial genetic factors become of more importance than nutritional factors during this stage, and may reflect a

familially inherited tendency for CHD which may be expressed as HC in early life.

With this evidence, what may be said about prevention? Firstly is CHD preventable? I view this pragmatically in that coronary heart disease, like obesity, is probably here to stay; but nonetheless I feel an attempt should be made to erode away the huge hidden bulk of causative factors. This leads on to primary versus secondary prevention (Oliver 1971; Miettenen et al 1972; Oliver et al 1978), but in this context I am concerned only with the former.

If one accepts the validity of primary prevention (Karvonen 1974; Stamler 1975), then to whom should these measures be directed, and how should they be implemented? One may use the known risk factor of HC as an example of the effects of prevention on a sample population. If one accepts that those with a serum cholesterol of greater than two standard deviations from the mean have an excess risk of CHD, then a decrease in the population level of serum cholesterol by one standard deviation would theoretically reduce this excess risk for 2.5% of the population, though it would not be possible to predict who would benefit (Whyte 1975). However, a similar shift downwards for those with FH, of whom half are at risk from early CHD, would theoretically reduce the risk for 47.5% of this sub-population. It would therefore be more efficient to direct specific preventive measures against those known to be at high risk, rather than the population as a whole. But how can those at high risk of early CHD be identified?

There are currently two screening methods in operation; firstly, the highly selective screening process carried out by family doctors

on subjects who present at the surgery, perhaps on the basis of the person's family history. There may be no quality control on BP recording done in the surgery, and the family doctors makes an individual decision as to when and if treatment should be started on for example, borderline hypertension or HC. This process is often carried out in a sickness-orientated environment in which the opportunity for health orientated counselling about regular physical exercise and not smoking may not be present.

The second method is that of population screening. This may be a self selected random screening such as occurs at the Sydney Hospital (Simons and Jones 1978), a whole community screening programme as at Busselton (Cullen 1972), or target population screening, for example on schoolchildren, or university students as is routinely done at Adelaide University. In America there have been three well known child population screening programmes; the Bogalusa Study in Louisiana, the Tecumseh Study, and the Muscatine Study. These were each enormously expensive research programmes looking into the prevalence of assumed coronary risk factors in children of all ages, and some of these data have been previously discussed.

Which method is most effective, and which worthwhile when considered as a practical measure in our community? The results of these studies described in this thesis included finding a high prevalence rate of HC in children, clustering of risk factors for CHD, and evidence of the early appearance of the profile which characterises the coronary-prone adult, and an actual decline in physical fitness through late adolescence. It is clearly difficult to unravel the relative importance of the environment and genetic

background in the emergence of these risk factors, and to decide at what age screening would become most effective both from a practical point of view as well as from a biological time point. This is the theoretical background to the problem of the detection and prevention of the antecedents of CHD in children: but what practical steps should be taken? Working papers from the WHO clearly state that the paediatrician has a role in the primary prevention of CHD precursors in children (WHO 1978). From my results a screening programme for hypercholesterolaemia would seem to be most likely to be effective done on primary school age children. By then biochemical patterns have been established, the children themselves would be amenable to both the testing and the surrounding health education, and the parents would also be more likely to be concerned and institute any necessary changes than for a teenager.

My conclusion of this question is that the problem has a socio-cultural base, and that although intensive programmes on small groups or individuals may be useful, and that individuals may reduce cardiovascular risk by exercise, diet, and positive attitudes to stress and mental health, the solution, or at least the route to the amelioration CHD, has a socio-political base. Health education in schools relating to nutrition, exercise, and smoking is not going to change unless it is made clear to the population as a whole, and politicians in particular, that the future health needs to be improved starting from preschool and primary school aged children.

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